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Note

Resolution of the enantiomers of propranolol and other beta-adrenergic antagonists by high-performance liquid chromatography

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The important β-adrenergic antagonist propranolol [1-isopropylamino-3-(1-naphthoxy)-2-propanol] is used clinically as the racemic mixture of the *R*-(+) and *S*-(−) isomers. The pharmacological properties of the enantiomers, however, are quite different, and the β-adrenergic blocking activity resides in the *S*-(−) isomer [1,2]. This observation has generated considerable interest in the chromatographic resolution of the enantiomers for analytical and preparative purposes. The most frequently used approach to this problem appears to be derivatization of the drug with a chiral reagent, followed by chromatographic resolution of the resulting diastereomers. Using gas—liquid chromatography (GLC), the propranolol enantiomers have been resolved as the derivatives formed with N-trifluoroacetyl-*S*-prolyl chloride (TPC) [3] or with *S*-(−)-1-phenylethyl isocyanate (PEIC) [4]. High-performance liquid chromatography (HPLC) has also been used for the resolution of propranolol, employing TPC [5,6], PEIC [4], *tert.*-butoxycarbonyl-L-alanine anhydride [7], or *tert.*-butoxycarbonyl-L-leucine anhydride [7] as the chiral derivatizing reagent. The latter two amino acid anhydrides have significant disadvantages in that they are not commercially available, and the derivatization procedure is rather elaborate. TPC also suffers from a disadvantage inasmuch as commercial samples contain 6—10% of the *R*-proline-derived enantiomer arising from partial racemization during the synthesis or storage of the reagent [6,8,9].

Other approaches to the chromatographic resolution of propranolol appear to be limited to an HPLC procedure using ion-pair chromatography with a chiral ion in the mobile-phase [10].

Other β-adrenergic antagonists related to propranolol in chemical structure have been studied less extensively. Alprenolol, oxprenolol, atenolol, pindolol,

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4-hydroxypropranolol, pronethalol and nifenalol have been resolved using a complex derivatization scheme based on TPC, followed by capillary GLC [3]. HPLC was applied to the resolution of the enantiomers of alprenolol and metoprolol, using the above-cited amino acid anhydrides [11], or with a chiral ion in the stationary phase [10].

In this communication, we describe a new and convenient HPLC method for the resolution of the enantiomers of propranolol and ten other, related, compounds.

EXPERIMENTAL

Chemicals and reagents

(−)-Alprenolol was purchased from Sigma (St. Louis, MO, U.S.A.). The following compounds were generously donated: (+)-alprenolol (Vega Biochemicals, Tucson, AZ, U.S.A.), (±)-propranolol, (−)-propranolol, (±)-pronethalol, and (±)-deacetylpractolol (Ayerst Labs., New York, NY, U.S.A.); (±)-4-hydroxypropranolol (Dr. John Thompson, School of Pharmacy, University of Colorado); (±)-metoprolol (CIBA Pharmaceutical, Summit, NJ, U.S.A.); (±)-pindolol (Mr. Allen Chapman, Pharmacy, University of Colorado Health Sciences Center); (−)-pindolol (Dr. Nancy Zahniser, School of Medicine, University of Colorado); (±)-sotalol and (±)-atenolol (Dr. John Gerber, School of Medicine, University of Colorado); (±)-bupranolol and (−)-bupranolol (Dr. Thomas Dunwiddie, School of Medicine, University of Colorado), and (±)-practolol (Dr. Joe Masserano, School of Medicine, University of Colorado).

The chiral reagents, 2,3,4-tri-O-acetyl-α-D-arabinopyranosyl isothiocyanate (AITC) and 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) were purchased from Polysciences (Warrington, PA, U.S.A.), acetonitrile (distilled-in-glass grade) from Burdick and Jackson Labs. (Muskegon, MI, U.S.A.), and ammonium phosphate monobasic from J.T. Baker (Phillipsburg, NJ, U.S.A.).

Chromatography

A Waters (Milford, MA, U.S.A.) HPLC system consisting of a Model M-45 and a Model M-6000 solvent delivery system, a Model U6K injector, a Model 440 absorbance detector, and a Model 660 solvent programmer was used. The Model M-45 solvent delivery system delivered acetonitrile, while the Model M-6000 pump delivered a 0.02 *M* aqueous ammonium phosphate solution. The two liquids were mixed in the M-6000 pump under the control of the solvent programmer. Mixtures of various compositions were used (Table I), and each mixture was delivered to the chromatographic column in the isocratic mode. Separations were carried out on a Beckmann Instruments (Berkeley, CA, U.S.A.) Ultrasphere ODS 5-μm 150 mm × 4.6 mm reversed-phase column. The column effluent was monitored at 254 nm. Chromatograms were recorded using a Hewlett-Packard (Avondale, PA, U.S.A.) Model 3380 A electronic integrator.

Preparation of derivatives

A 1-mg sample of the compound to be derivatized, in the free base form, was

TABLE I

RESOLUTION OF β-ADRENERGIC ANTAGONISTS WITH AITC OR GITC

Chromatographic and derivatization conditions are given in the Experimental section.

Compound	Retention time (min) of diastereomers* formed with				Percent acetonitrile in mobile phase**		Separation factor*** (α)	
	GITC		AITC					
					GITC	AITC	GITC	AITC
Propranolol	8.4 *S*-(−)	10.5 *R*-(+)	8.7 *R*-(+)	9.8 *S*-(−)	58	56	1.28	1.14
Pindolol	8.6 *S*-(−)	10.4 *R*-(+)	6.5 *R*-(+)	7.0 *S*-(−)	47	47	1.24	1.09
Alprenolol	6.4 *S*-(−)	7.7 *R*-(+)	5.8 *R*-(+)	6.5 *S*-(−)	65	65	1.24	1.15
Bupranolol	4.3 (−)	5.0 (+)	4.0 (+)	4.3 (−)	75	75	1.21	1.10
Atenolol	5.3	6.4	4.4	4.7	40	37	1.26	1.09
Pronethalol	6.8	8.7	6.8	8.1	58	56	1.28	1.22
Metoprolol	4.8	5.7	4.8	5.2	58	56	1.24	1.11
4-Hydroxypropranolol	9.2	11.3	6.9	7.5	50	50	1.26	1.10
Practolol	7.9	9.8	5.9	6.5	40	37	1.28	1.12
Deacetylpractolol	7.9	9.8	6.4	7.1	40	37	1.28	1.13
Sotalol	7.3	8.7	5.4	5.9	44	44	1.22	1.11

*When known, the identity of the drug enantiomer from which the diastereomer derives is given.
**Remainder of mobile phase aqueous ammonium phosphate as described in the Experimental section.
***Separation factor, defined in ref. 12.

treated with 200 μl of acetonitrile containing 2 mg of AITC or GITC in a test tube. The tube was tightly capped, swirl-mixed (vortex), and allowed to stand at room temperature for 30 min. Aliquots (5—10 μl) were then injected into the HPLC system.

RESULTS AND DISCUSSION

The chiral reagents GITC and AITC were recently described and applied to the HPLC resolution of enantiomeric amino acids [13,14] and the enantiomers of epinephrine and norepinephrine [15]. The isothiocyanato group reacts rapidly and selectively with primary and secondary amines under mild conditions to form the corresponding thiourea derivatives [13–15]. Since propranolol and related β-adrenergic antagonists are, in general, secondary amines, it appeared worthwhile to examine the applicability of GITC and AITC to the resolution of the enantiomers of these drugs. The results are collected in Table I. The enantiomers of each compound in Table I were well resolved as their GITC derivatives by reversed-phase HPLC; indeed, base-line resolution was achieved in each case, and the chromatographic peaks were remarkably narrow. Representative examples are shown in Fig. 1. The separation factor, α (Table I), remained surprisingly constant within the GITC series considering the wide variation in structure represented by the compounds studied. Individual enantiomers were available for four of the compounds, permitting the identification of the elution order of the diastereomeric derivatives. The diastereomer derived from GITC and the *S*-(−) enantiomer of propranolol, pindolol or alprenolol elutes before the diastereomer from the corresponding *R*-(+) enantiomer (Table I). The absolute configuration of bupranolol has not been published, but the levorotatory enantiomer of this drug also provided a GITC derivative with a shorter retention time than that of the diastereomer derived from (+)-bupranolol.

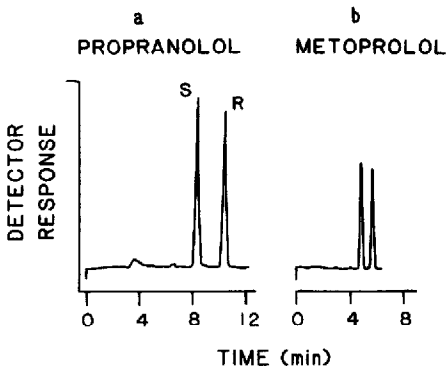

Fig. 1. Resolution of the enantiomers of (a) propranolol and (b) metoprolol as their GITC derivatives. The chromatographic conditions are given in the text and in Table I.

The diastereomers formed from AITC were in general considerably less-well resolved than the corresponding GITC derivatives (Table I). AITC is therefore not recommended for the separation of the enantiomers of the compounds listed in Table I. Interestingly, the order of elution of the diastereomers from AITC was the reverse of the order of elution of the GITC derivatives: the product from the reaction of AITC and the dextrorotatory drug elutes first in each instance (Table I). Such reversal of elution order was previously observed in the chromatography of the AITC and GITC derivatives of amino acids and catecholamines [13—15], and it was suggested that this phenomenon may be related to conformational differences between the two acetylglucosyl residues, especially at the anomeric carbon atom [14].

The procedure has several major advantages over previously described techniques: the derivatization is extremely simple; the chiral reagent is commercially available and is chemically and stereochemically stable; and baseline resolution of the diastereomers is achieved within a remarkably short analysis time. The procedure for the resolution of the enantiomers was not applied to the analysis of these compounds in biological fluids, but it appears that the technique may be adaptable to this purpose, since thiourea derivatives appear to be very sensitive to UV detection [13,14]. Further study will be required to confirm this expectation.

In conclusion, derivatization of propranolol and other β-adrenergic antagonists with GITC is a useful technique for the separation of their enantiomers by HPLC.

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