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R-α-METHYLBENZYL ISOTHIOCYANATE, A NEW AND CONVENIENT CHIRAL DERIVATIZING AGENT FOR THE SEPARATION OF ENANTIOMERIC AMINO COMPOUNDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

R-α-Methylbenzyl isothiocyanate (*R*-AMBI), a commercially available chiral compound, was evaluated as a chiral derivatizing agent for the separation of enantiomeric amines by reversed-phase liquid chromatography. Ephedrine, propranolol, phenylglycine, phenylglycinol, phenylalanine methyl ester and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane were derivatized with *R*-AMBI, and the derivatives chromatographed on octadecylsilane columns using aqueous methanol or acetonitrile as mobile phase. The separation factor, α, for the resolution of the enantiomers ranged between 1.05 and 1.24, and peak resolution, *R*, ranged between 1.29 and 3.28. In some applications, *R*-AMBI may have advantages over other similar reagents.

INTRODUCTION

The separation of enantiomers by high-performance liquid chromatography (HPLC) has been the subject of numerous investigations in recent years[1–10]. One frequently used approach to this problem is derivatization of the enantiomeric mixture with a chiral reagent, followed by chromatographic separation of the product diastereomers[6–10]. Numerous chiral derivatizing reagents have been used for this purpose[6–10]. One of these, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (TAGIT), is highly useful for the resolution of a variety of amino compounds[10–15]. Difficulties in obtaining TAGIT from commercial sources during a recent period prompted us to search for an alternative reagent with resolution capabilities similar to those of TAGIT[16]. A result of this search was the finding that *R*-α-methylbenzyl isothiocyanate (*R*-AMBI, Fig. 1) is a useful chiral derivatizing agent in HPLC.

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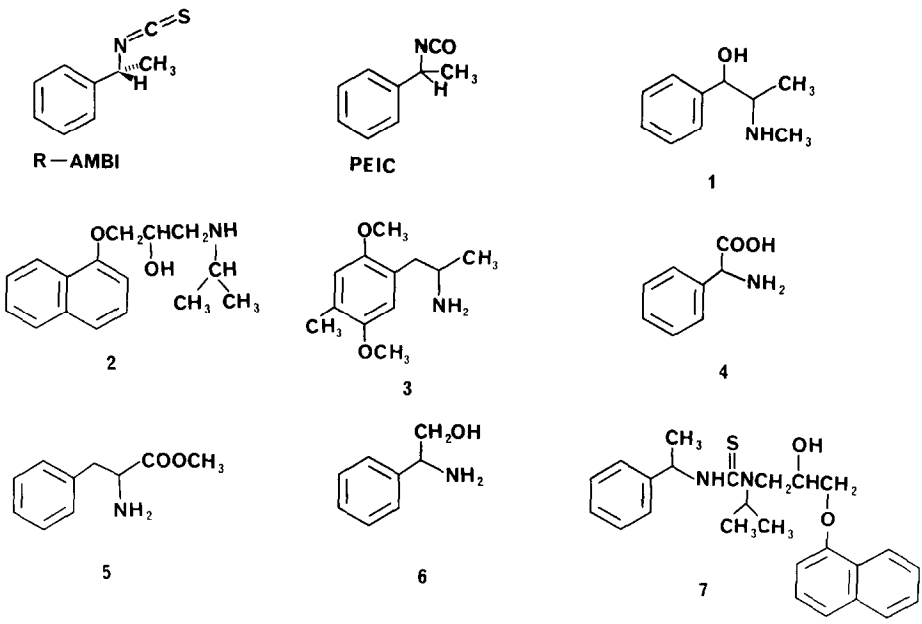

Fig. 1. Chemical structures of the compounds studied.

EXPERIMENTAL

Chemicals

(±)-Ephedrine hydrochloride, (−)-ephedrine, (−)-phenylglycinol, (±)- and (−)-phenylglycine were obtained from Aldrich (Milwaukee, WI, U.S.A.); 2-aminoethanol, (±)- and L-phenylalanine methyl ester hydrochloride from Sigma (St. Louis, MO, U.S.A.); acetonitrile, dichloromethane and methanol, distilled-in-glass grade, were from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.); (±) and (+)-propranolol hydrochloride from Ayers Labs. (New York, NY, U.S.A.); ammonium phosphate monobasic, sodium hydrogen carbonate, and triethylamine were obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.); (±)- and (−)-1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) hydrochloride were generous gifts from Dr. Neal Castagnoli, University of California (San Francisco, CA, U.S.A.); DL-and D-α-methylbenzyl isothiocyanate were purchased from Trans World Chemicals (Kensington, MD, U.S.A.).

Derivatization procedures

The following procedure was generally used, except in special cases noted below: 1 mg of the amine in the free base form in a test tube was treated with 200 μl acetonitrile containing 2 mg of *R*-AMBI. The tube was swirl-mixed (Vortex) and allowed to stand at room temeprature for 30 min. Acetonitrile (200 μl) was added, the tube was briefly Vortex-mixed, and aliquots (2–4 μl) were injected into the HPLC system.

Phenylalanine methyl ester hydrochloride (0.5 mg) was treated with 100 μl of saturated sodium bicarbonate solution and 2.5 ml dichloromethane in an extraction

tube. The mixture was Vortex-mixed for 30 sec, centrifuged, and the aqueous layer was aspirated and discarded. The organic layer was transferred to another tube and the solvent evaporated under a stream of nitrogen. The residue was treated with 100 μl acetonitrile containing 1 mg of the derivatizing agent. After 30 min at room temperature, acetonitrile (100 μl) was added and HPLC analysis was performed.

Phenylglycine (5 mg) was dissolved in 2 ml 50% aq. acetonitrile containing 10 μl of triethylamine. A 100-μl aliquot of the solution was treated with 200 μl of acetonitrile containing 1 mg of the chiral reagent. After 30 min, HPLC analysis was carried out.

In some cases, 2 μl of 2-aminoethanol were added to the reaction mixture at the end of the 30-min derivatization period in order to destroy the excess *R*-AMBI remaining. Chromatographic analysis was performed after 20 min.

Derivatization time course study

(±)-Ephedrine (1.0 mg) was placed in a test tube. The chiral reagent (2.0 mg) dissolved in 200 μl acetonitrile, was added and the tube was Vortex-mixed for 10 sec. At appropriate times, 2-μl aliquots were withdrawn and were added to 100 μl of the mobile phase in a test tube. Aliquots (25 or 50 μl) of the resulting solution were injected into the HPLC system.

Chromatography

A Waters Assoc. (Milford, MA, U.S.A.) HPLC system consisting of a Model M-6000 solvent delivery system, a Model U6K injector, and a Model 440 absorbance detector was used. Separations were carried out on a Beckmann (Berkeley, CA, U.S.A.) 150 × 4.6 mm I.D. column packed with Ultrasphere ODS of 5-μm particle size. The mobile phases used (Table I) were prepared by first vacuum-filtering the individual components and then mixing in the appropriate ratio. The mobile phase

TABLE I

SEPARATION OF ENANTIOMERIC AMINES AS THEIR DERIVATIVES FORMED WITH *R*-α-METHYLBENZYL ISOTHIOCYANATE

Compound		*Mobile phase***	α***	*R*§	t_R *(min)*
*No.**	*Name*				
1	Ephedrine	50:50 A–W	1.20 (1.19)[14]	3.28 (2.73)[14]	7.8 (−); 9.1 (+)
2	Propranolol	65:35 A–W	1.15 (1.28)[13]	2.67	12.0 (+); 13.6 (−)
2	DOM	65:35 M–W	1.08	1.88 (1.54)[15]	24.4 (−); 25.1 (+)
4	Phenylglycine	40:60 M–B	1.24 (1.33)[10]	2.48 (2.80)[10]	12.2 (+); 14.8 (−)
5	Phenylalanine methyl ester	57.5:42.5 M–B	1.05	1.29	20.7 (+); 21.8 (−)
6	Phenylglycinol	50:50 M–B	1.10	2.06	21.7 (+); 23.8 (−)

* Refers to Fig. 1.

** Volumes of the two components were mixed in the ratios indicated. A = acetonitrile, B = 0.02 *M* monobasic ammonium phosphate, M = methanol, W = water. Other chromatographic conditions are given in the Experimental.

*** Separation factor[17]. The figures in parentheses are published values obtained using TAGIT for the separation of the enantiomers in the references indicated.

§ Peak resolution[18]. The figures in parentheses are published values obtained using TAGIT in the references indicated.

was delivered at 1.0 ml/min, and the column effluent was monitored at 254 nm. The detector output was recorded using a Perkin-Elmer (Norwalk, CT, U.S.A.) Model 56 strip-chart recorder.

RESULTS

The time course of the derivatization of ephedrine with *R*-AMBI is shown in Fig. 2. The reaction is complete in *ca.* 30 min. A similar time course was observed in the derivatization of other amines (data not shown), and therefore in routine derivatizations a 30-min reaction time was used.

The chromatographic resolution of six racemates is summarized in Table I. Fig. 3 shows the resolution of the enantiomers of propranolol.

The enantiomeric purity of *R*-AMBI was determined by derivatizing the reagent with optically pure (−)-ephedrine. Chromatographic analysis of the product revealed that the chiral reagent contained 1.8% of the *S*-enantiomer.

Excess *R*-AMBI in the reaction mixture eluted with a t_R which depended on the composition of the mobile phase. Under the chromatographic conditions employed for the derivatives of ephedrine, *R*-AMBI had a t_R of 16.2 min *i.e.*, longer than the t_R of the ephedrine derivatives. When the derivatives of propranolol were chromatographed, on the other hand, the t_R of *R*-AMBI was 6.7 min, *i.e.*, shorter than the t_R of the propranolol derivatives (Fig. 3). Excess *R*-AMBI in the reaction mixture could be converted via reaction with 2-aminoethanol to a derivative with a

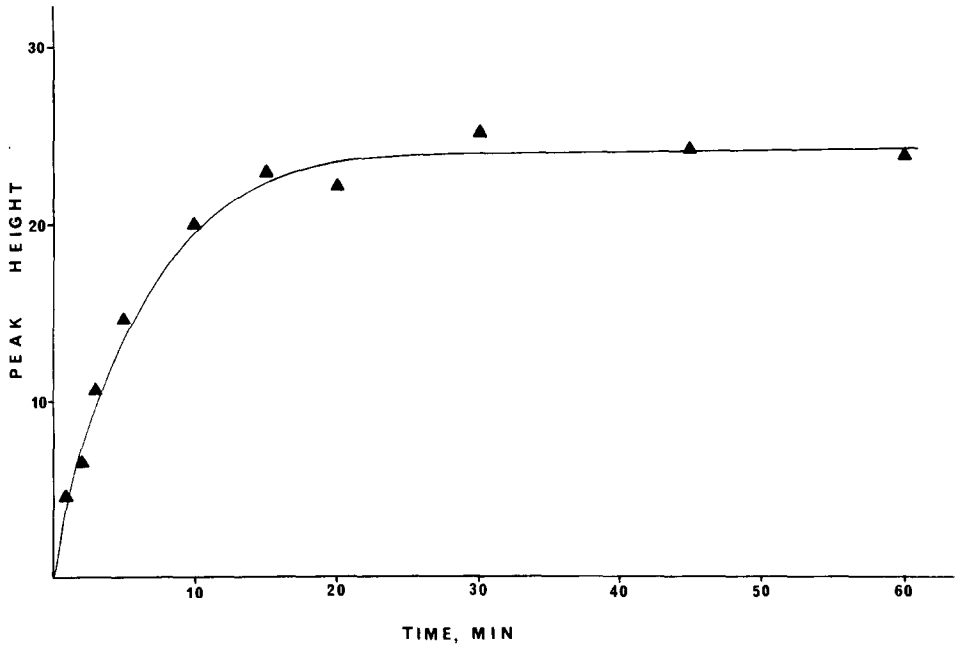

Fig. 2. Time-course of the reaction of (±)-ephedrine with *R*-AMBI under conditions given in Experimental. The data shown are for the (−)-ephedrine-derived peak; the time course for the formation of the (+)-ephedrine derivative was essentially identical.

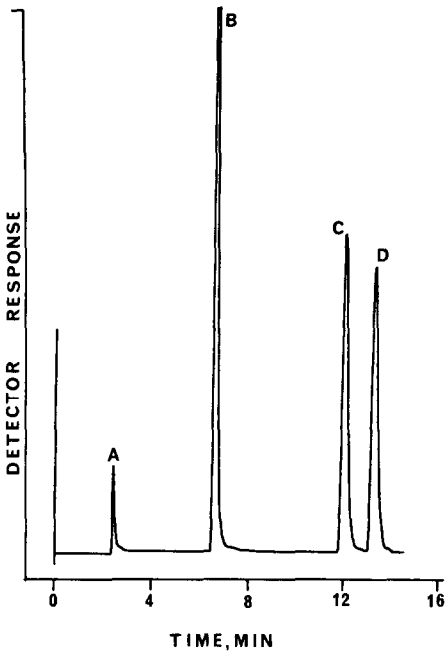

Fig. 3. HPLC resolution of (±)-propranolol after reaction with *R*-AMBI. Chromatographic conditions are given in Experimental and in Table I. Peak identification: A, unidentified; B, excess *R*-AMBI; C, derivative of (+)-propranolol; D, derivative of (−)-propranolol.

short t_R. For this purpose, 2-aminoethanol was added at the end of the derivatization period, and 20 min was allowed for its reaction with *R*-AMBI. In the resolution of ephedrine, the reaction product of 2-aminoethanol with *R*-AMBI had a t_R of 2.3 min.

DISCUSSION

TAGIT, an isothiocyanate, reacts with enantiomeric primary or secondary amines to give the corresponding diastereomeric thioureas which in many cases were readily separated by HPLC[10–15]. The derivatization reaction is rapid and selective, and takes place under mild conditions. It seemed reasonable, therefore, to focus on other isothiocyanates in our search for alternatives to TAGIT. Nambara *et al.*[19] described the application of (+)-neomenthyl isothiocyanate and (−)-1,7-dimethyl-7-norbornyl isothiocyanate to the HPLC resolution of the racemates of several amino acids. To obtain adequate resolution, the thiourea derivatives had to be converted to their *tert.*-butyldimethylsilyl ester derivatives, and normal-phase chromatography was required for the separation of the diastereomeric derivatives[19]. The procedure was not applied to other types of amino compounds, and the two chiral isothiocyanates are not available commercially. No other chiral isothiocyanates appear to have been used in the chromatographic resolution of racemates.

R-AMBI (Fig. 1) is a chiral compound available as D-α-methylbenzyl isothiocyanate from a commercial supplier [a sample of D-α-methylbenzyl isothiocyanate purchased from ICN/K&K Laboratories, Inc. (Plainview, NY, U.S.A.) proved to be

the racemix mixture]. To determine the enantiomeric purity of *R*-AMBI, the reagent was reacted with (−)-ephedrine, and the product analyzed by HPLC. The (−)-ephedrine sample used was enantiomerically pure [>99.8%; no (+)-ephedrine detected] as determined using a previously developed method[14]. It was found that the *R*-AMBI sample used in this work contained 1.8% of the *S*-antipode. This may render the chiral reagent unsuitable for some applications, but such a low level of enantiomeric contamination may be acceptable for other purposes, and the extent of contamination can be determined accurately using (−)-ephedrine.

Under the derivatization conditions employed, the reaction of *R*-AMBI with the amines was complete in *ca.* 30 min, and an aliquot of the reaction mixture could be directly injected into the HPLC system. While the presence of excess derivatizing agent in the reaction mixture posed no problem in our studies, it may be objectionable in some applications, inasmuch as the reagent peak may overlap with a peak of interest, or may have an unduly long t_R, resulting in an inconveniently long analysis time. To avoid such problems, 2-aminoethanol can be used to destroy excess *R*-AMBI.

The resolution of six racemates was studied (Table I). These compounds encompass a reasonable variation in structure (Fig. 1), and included an amino acid (4), an amino acid ester (5), amino alcohols (1, 2, 6), and an arylalkylamine (3). Both primary (3–6) and secondary (1, 2) amino groups were represented. With the exception of the resolution of phenylalanine methyl ester, the separation of the diastereomeric derivatives was excellent, as judged by the values of the resolution factor *R* (Table I), inasmuch as the separation of two equal-sized peaks is essentially complete when $R = 1.5$ (ref. 18). The chemical structure (7) of the adduct between *R*-AMBI and propranolol is shown in Fig. 1, and the chromatographic separation of the diastereomers is shown in Fig. 3.

The mobile phases used were mixtures of water or aq. ammonium phosphate and methanol or acetonitrile. It was found that in some cases, *e.g.*, compound 3, methanol instead of acetonitrile in the mobile phase provided a drastically improved separation of the diastereomeric *R*-AMBI derivatives (data not shown). Interestingly, however, replacing acetonitrile with methanol (at a higher concentration) in the analysis of the ephedrine derivatives did not result in a significant improvement in resolution.

It is interesting to compare the resolutions obtained with *R*-AMBI to those obtained with TAGIT, a comparison which can be made for four of the six compounds studied. It can be seen from Table I that *R*-AMBI gave greater resolution for ephedrine and DOM, but TAGIT was better for the resolution of phenylglycine and propranolol. It is noteworthy in this context that *R*-AMBI costs *ca.* 50 times less than TAGIT. It is also interesting to compare *R*-AMBI to its oxygen analogue, 1-phenylethyl isocyanate (PEIC, Fig. 1). The *R* form of PEIC was used by Thompson *et al.*[9] for the HPLC resolution of propranolol. The authors did not give the α or *R* values for the HPLC separation of the derivatives, but visual comparison of the chromatogram of the PEIC derivatives[9] to that of the *R*-AMBI derivatives (Fig. 3) of propranolol clearly indicates that *R*-AMBI provides significantly better resolution in a shorter time. Another disadvantage of PEIC is its chemical instability[20], which does not appear to be a problem with *R*-AMBI[21].

In summary, *R*-AMBI is a commercially available chiral reagent which appears

to be useful for the reversed-phase HPLC separation of the enantiomers of a variety of amines. The elucidation of the full scope of applications of *R*-AMBI remains for future investigations.

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