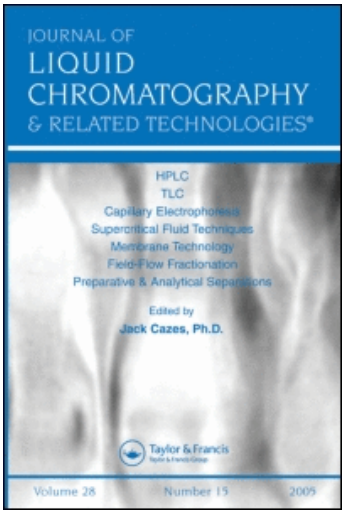

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Zhou, Ye , Sun, Zeng Pei and Lin, Da Kui(1990) 'Liquid Chromatographic Evaluation of a New Chiral Derivatizing Reagent for Enantiomeric Resolution of Amine and Alcohol Drugs', Journal of Liquid Chromatography & Related Technologies, 13: 5, 875 — 885
To link to this Article: DOI: 10.1080/01483919008049219
URL: http://dx.doi.org/10.1080/01483919008049219

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(5), 875-885 (1990)

LIQUID CHROMATOGRAPHIC EVALUATION OF A NEW CHIRAL DERIVATIZING REAGENT FOR ENANTIOMERIC RESOLUTION OF AMINE AND ALCOHOL DRUGS

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ABSTRACT

A new chiral resolving reagent N-(p-toluenesulfonyl)prolyl isocyanate(TSPI) was prepared with a very practical method and evaluated for liquid chromatographic resolution of amine and alcohol enantiomers via diastereomeric derivatization. This procedure is rapid and convenient, with good separation, few by-products. Expected structures of the derivatives were confirmed by mass spectra. Ordinary normal phase and the mobile phase of petroleum ether were used.

INTRODUCTION

Liquid chromatography has been greatly employed in the separation of a variety of enantiomers. Although

more studies on direct resolution of enantiomers by HPLC have been reported recently, using chiral stationary phase and chiral mobile phase, the indirect separation with achiral chromatography system via diastereomeric derivatization is still widely used in common laboratory.

Chiral isocyanate is usually considered to be a kind of the most useful resolving reagent. Only S(-) or R(+)-naphthalylethyl isocyanate(NEIC) and S(-) or R(+)-phenylethyl isocyanate(PEIC) have been reported so far(1,2). Both of them were synthesized from their corresponding optical active amines by the action of phosgene on the hydrochloride of amine or by treatment of ethyl carbamate of amine with trichlorosilane(3). However, their synthetic material sources were expensive and not easy to get, additionally, phosgene was very toxic and the procedure was not practical in ordinary analysis laboratory.

On the other hand, chiral reagents were often prepared from L-amino acids which were the most common compounds of high optical purities. Of all amino acids, L-proline is employed most frequently by virtue of the principle that optical resolution might be greatly increased and the configuration of the chiral carbon atom might be best kept if the asymmetric center is part of a ring system(4). However, so far the chiral reagents

prepared from L-proline were usually activated acids such as acid chlorides instead of isocyanates.

Therefore, with a practical synthetic route, from L-proline, we have prepared two chiral isocyanates, i.e. N-(p-toluenesulfonyl)prolyl isocyanate(TSPI) and N-((2-naphthalene)sulfonyl)prolyl isocyanate(NSPI). They have similar ability of optical separation, and NSPI will be described in my another paper in press. The work herein reported the synthesis and liquid chromatographic evaluation of TSPI for indirect separation of amine and alcohol enantiomers.

EXPERIMENTAL

Chemicals and reagents

L-proline and sodium azide were obtained from Sigma and Aldrich, respectively. Ethyl chloroformate(CP), triethylamine(AR), p-toluenesulfonyl chloride(CP) and other solvents(AR) were purchased from domestic market.

Racemic mexiletine hydrochloride, amphetamine and phenylpropanol are all used as drug materials. The free base of mexiletine was obtained from its salt by adding aqueous ammonia and then extracting with chloroform.

Chromatography

The HPLC system consisting of a Waters Model 6000A pump, a Rheodyne 7105 injector, a Shimadzu SPD-1 UV de-

tector coupled with a Hitachi 056 recorder or a Shimadzu C-R3A Chromatopac was used.

Separation was carried out on a 30cm*4.0mm I.D. column(Waters) packed with silica gel of 7-9μm particle size(tianjing, China). The detection was mornitored at 254nm. The mobile phase was consisted of petroleum ether and isopropanol in appropriate proportions at flow-rate of 1.5ml/min.

Synthesis of the chiral reagent

N-(p-toluenesulfonyl)proline(TSP) was prepared from L-proline and P-toluenesulfonyl chloride, referring to the previous paper(5) .

N-(p-toluenesulfonyl)prolyl azide(TSP-N_3) was synthesized from TSP, ethyl chloroformate and sodium azide, with the method similar to synthesization of cis-2-phenylcyclopropyl azide(6). IR spectrum(in KBr): 2160, 1720 cm^{-1} peaks denoted the azide group.

TSP-N_3 is stable to storage in refrigerator, survives melting, but loses nitrogen at approximately 100 °C to yield TSPI, consulting the Pirkle's paper(7). Because isocyanate is sensitive to moisture, it is important to convert TSP-N_3 into TSPI just before derivatization. IR spectrum(in KBr): 2280 cm^{-1} peak denoted the isocyanate group. Mass spectrum(EI) with molecular ion peak at m/z 266 was observed.

Derivatization

A 10% excess of TSP-N_3 in toluene was heated under reflux for 10min. the racemic amine was then added to this solution. The reaction mixture was kept at room temperature for 10min, then chromatographed without any work up beyond cooling and dilution. For racemic mexiletine, the resulting derivative could precipitate from the solution. The precipitate was filtered and determined by mass spectrometer(EI). The molecular ion peak at m/z 445 was afforded.

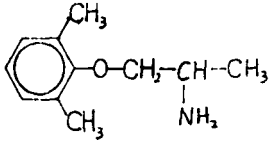
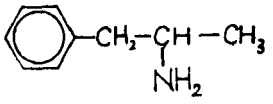
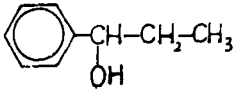
Racemic alcohol drug was derivatized with above method, but the reaction mixture was heated at 100 °C for 1h, instead of keeping at room temperature. To affirm the structure of the derivative, the reaction solution was washed with 0.1% NaOH, 0.1% HCl and water respectively, then dried over anhydrous sodium sulfate and the organic layer was evaporated to dryness under nitrogen stream. Mass spectrum of resulting residue showed a molecular ion peak at m/z 402, which suggested the expected carbamate.

RESULTS AND DISCUSSION

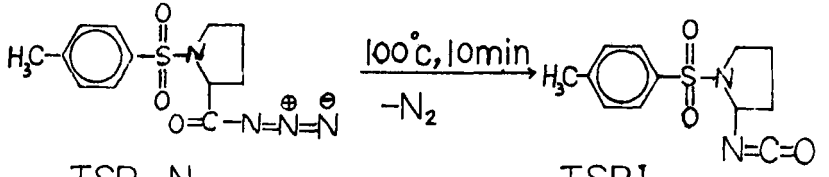
It is very important for indirect resolution of enantiomers by HPLC that the chiral resolving agent has high optical purity and no racemization occurred in the process of derivatization as well as chromatography.

TABLE 1

Chromatographic Results for the Diastereomers Derived From the Amine and Alcohol Enantiomers with TSPI Reagent

Compound and structure	k_1'	k_2'	α	R_s	i-prOH% in mobile phase
Mexiletine 	8.52	13.2	1.54	4.35	3
Amphetamine 	8.90	11.2	1.26	1.21	3.5
Phenylpropanol 	13.6	16.3	1.20	4.22	1

* k_1', k_2': the capacity factors of the first-eluted and the second-eluted enantiomers, respectively.



TSP-N_3 TSPI

** Chemical structures of the chiral reagents

L-proline is the only L-amino acid that its chiral center is on a ring system of high configuration immobility, except L-hydoxyproline. On the other hand, it has been concluded that the formation of oxazolone intermediate is the major cause of racemization of activated N-acetyl amino acid(8). Owing to having no protons on the nitrogen atom to form oxazolone intermediate, N-protected proline is the most suitable L-amino acid for synthesizing a chiral resolving reagent. In addition, in TSP-N_3 molecule, the attraction between the negative charge developing on the amide oxygen and the positive charge on the central nitrogen atom of its azide group inhibits the bond movements which are necessary to attain the conformation needed for oxazolone formation(9). Therefore, we storaged TSP-N_3 reagent in refrigerator till derivatization, which could best keep the optical purity of the chiral reagent and avoid the isolation of TSPI reagent.

Besides, L(-)1-phenylethylamine(optical purity of 99%, Merck-Schuchardt) was applied to react with TSPI in the same way. No obvious chromatographic peak of the enantiomeric impurity of TSPI was observed in Figure 3, which further demonstrated that no racemization occurred in whole process of the separation of amine isomers. Additionally, the condition used for derivatizing the alcohol was more vigorous than that for amine. Unfortu-

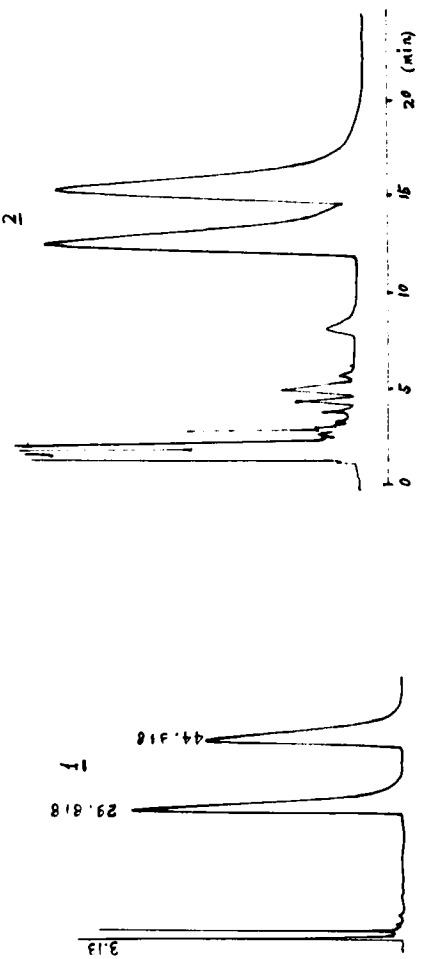


FIGURE 1. Separation of the OSPI derivatives of (a)racemic mexiletine, 1; (b) racemic amphetamine, 2.

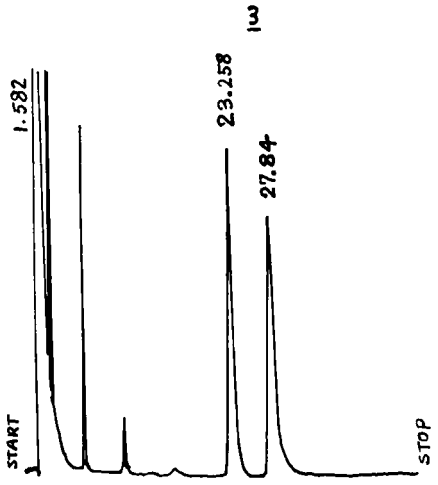


FIGURE 2. Separation of the TSPI derivatives of racemic phenylpropanol, 3.

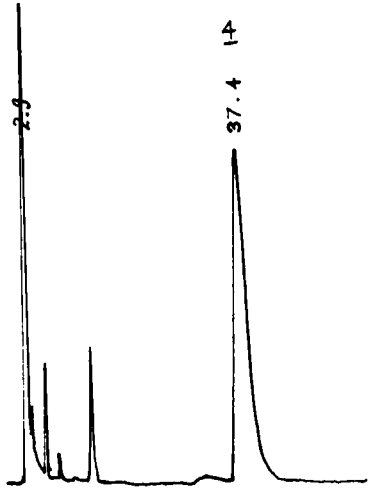


FIGURE 3. Chromatogram representing the TSPI derivative of L(-)1-phenylethylamine, 4. Mobile phase, petroleum ether:isopropanol (100:2); flow-rate, 1.0ml/min.

nately, we have no alcohol compound of high optical purity so that we have not known if the racemization might occur during the derivatization of alcohol. Extensive work will be in progress.

The results indicated that as a chiral resolving reagent, TSPI has several desirable properties. It was inexpensive and practical to synthesize TSPI of high optical purity. The derivatization was convenient to operate and produced few by-products. Good separations were afforded for chiral amine and alcohol drugs. Especially for resolving alcohol enantiomers, TSPI had greatly shortened the derivatization time, while NEIC had been reported to resolve racemic 3-O-hexadecylglycerol after derivatizing at 80 °C for 36hr(2). It may be one of the reasons that the central carbon atom in isocyanate group of TSPI is more positive and results in more reactive than the one of NEIC because the nitrogen atom in proline of TSPI can attract electrons while the naphthalene group of NEIC can repel electrons. On the other hand, the larger groups connecting with the chiral carbon atom of 3-O-hexadecylglycerol may reduce the reactivity of its hydroxy group with isocyanate of NEIC. Mass spectra data of the derivatives confirmed their expected structures that the isocyanate formed urea with amine and carbamate with alcohol. In conclusion, TSPI may become a versatile and potential chiral derivatizing reagent.

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