Journal of Chromatography, 508 (1990) 220–224
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 312

Note

Liquid chromatographic evaluation of chiral derivatizing reagents for the resolution of amine and alcohol enantiomers

YE ZHOU* and ZENG-PEI SUN
National Institute for the Control of Pharmaceutical and Biological Products, Beijing 100050 (China)
(First received September 12th, 1989; revised manuscript received January 15th, 1990)

Chiral isocyanates are usually considered to be one of the best derivatizing reagents for the indirect separation of drug enantiomers. Only two compounds of this kind, *viz.*, *S*(−)- or *R*(+)-naphthylethyl isocyanate (NEIC) and *S*(−)- or *R*(+)-phenylethyl isocyanate (PEIC), have been reported so far. Both of them give good resolutions of optical amines[1,2], but only NEIC has been reported to resolve two enantiomeric alcohols[3,4] on heating at 80°C for 36 h during derivatization. Further, these isocyanates were prepared from their corresponding optical amines by the action of phosgene or trichlorosilane, which are toxic and not practical in an ordinary analytical laboratory.

Therefore, we first synthesized N-[(2-naphthalene)sulphonyl]prolyl isocyanate (NSPI) from L-proline. This paper describes its synthesis and liquid chromatographic evaluation for the optical separation of amines and alcohols, and the structural identification of their diastereomeric derivatives.

Moreover, an azide is usually applied as an intermediate in the synthesis of isocyanates, to conjugate with the amino group of an amino acid to form peptides in organic chemistry, having the advantage of minimum racemization[5]. We therefore need N-[(2-naphthalene)sulphonyl]prolyl azide (NSP-N_3) to resolve amine enantiomers, which has not been reported previously. This procedure retains the configurations of the chiral centres during derivatization, which is very important for the indirect separation of enantiomers.

EXPERIMENTAL

Chemicals and reagents

L-Proline (Sigma, St. Louis, MO, U.S.A.) and 2-naphthalenesulphonyl chloride (Fluka, Buchs, Switzerland) were used. Ethyl chloroformate, triethylamine, light petroleum (b.p. 60–90°C), isopropanol, toluene and other chemicals of analytical-reagent grade were purchased in China.

Racemic mexiletine hydrochloride, amphetamine and 1-phenylpropanol were obtained as drug materials. The free base of mexiletine was obtained from its hydrochloride.

0021-9673/90/$03.50

Apparatus

The high-performance liquid chromatographic (HPLC) system consisted of a Waters Model 6000A pump, a Rheodyne 7105 injector and a Shimadzu SPD-1 UV detector monitoring at 254 nm, coupled with a Hitachi 056 recorder or a Shimadzu Chromatopac C-R3A.

The stationary phase was silica gel (7-9 μm) (Tianjing, China) packed in a 30 cm × 4.0 mm I.D. column by the slurry packing method. The mobile phase was a mixture of light petroleum and isopropanol in suitable proportions.

Preparation of chiral derivatizing reagents

S(−)-N-1-(2-Naphthalenesulphonyl)-2-proline (NSP) was prepared from L-proline and 2-naphthalenesulphonyl chloride as described previously[6] and had m.p. 130–133°C.

N-[(2-Naphthalene)sulphonyl]prolyl azide (NSP-N_3) was synthesized from NSP, ethyl chloroformate and sodium azide[7]. In the IR spectrum (KBr), peaks at 2160 and 1720 cm^{-1} denoted the azide group.

N-[(2-Naphthalene)sulphonyl]prolyl isocyanate (NSPI) was prepared by heating NSP-N_3 at 80°C for 10 min, according to Pirkle et al.[8]. In the IR spectrum (KBr), the peak at 2280 cm^{-1} denoted the isocyanate group; mass spectrum (electron impact), *m/z* 302 ($M^{+\cdot}$).

To test the optical purities of the NSPI and NSP-N_3 reagents, L-(−)-1-phenylethylamine (optical purity 99%; Merck-Schuchardt) was made to react with the two reagents. No obvious chromatographic peaks of enantiomeric impurities were observed.

Synthesis of urea and carbamate with NSPI

Isocyanate is sensitive to moisture, whereas azide is comparatively stable. Therefore, we kept NSP-N_3 in a refrigerator and converted it into NSPI just before derivatization.

A solution of NSP-N_3 (0.01 mmol) in 1 ml of toluene was heated at 80°C for 10 min in a 5-ml reaction vial equipped with a reflux condenser and a calcium chloride drying tube. Racemic amine drug (0.01 mmol) was added to the above solution. The reaction mixture was kept at room temperature for 10 min, then chromatographed.

The racemic alcohol drug was reacted as above but instead of standing at room temperature the reaction solution was heated at 80°C for 1 h.

Synthesis of amide diastereomers with NSP-N_3

To a solution of NSP-N_3 (0.01 mmol) in 1 ml of toluene, an equivalent amount of racemic amine drug was added. The mixture was kept at room temperature for 10 min and then directly chromatographed.

Identification of the derivatives by mass spectrometry

Each of the above reaction solutions was mixed with chloroform and then washed with 0.1% NaOH, 0.1% HCl and water. The organic layer was dried over anhydrous sodium sulphate, then evaporated to dryness and the mass spectrum was obtained with electronic impact ionization.

TABLE I

SEPARATION AND MASS SPECTRUM IDENTIFICATION OF NSPI AND NSP-N_3 DERIVATIVES OF AMINE AND ALCOHOL ENANTIOMERS

k'_1 and k'_2 = capacity factors of the first- and second-eluted enantiomers, respectively; $M^{+\cdot}$ = molecular weight obtained by mass spectrum (electron impact).

Compound and structure	*NSPI*					*NSP-N_3*				
	k'_1	k'_2	α	R_s	$M^{+\cdot}$	k'_1	k'_2	α	R_s	$M^{+\cdot}$
Mexiletine	5.70	9.11	1.6	3.50	481	20.3	23.2	1.14	2.70	466
Amphetamine	10.7	13.8	1.29	2.31		2.47	3.35	1.36	3.2	422
1-Phenylpropanol	11.0	12.7	1.16	2.4	438					

Comparison between NSP-N_3 and NSPI for enantiomeric separation of amines

A solution of NSP-N_3 in toluene was heated at 60°C for 10 min so that only part of NSP-N_3 was converted into NSPI. An equivalent amount of racemic mexiletine was added. The reaction mixture was kept at room temperature for 10 min, then two pairs of diastereomers of NSP-N_3–mexiletine and NSPI–mexiletine were obtained and chromatographed (Fig. 5).

RESULTS AND DISCUSSION

The mass spectra showed that the isocyanate formed ureas with amines and carbamates with alcohols whereas the azide formed amides with amines.

The results in Table I demonstrated that the procedure, for the indirect separation of amine and alcohol isomers using NSPI has several advantages, such as simple and rapid operation, good resolution, the derivatives are chromatographed directly without clean-up, and with high UV absorbance and strong fluorescence. In

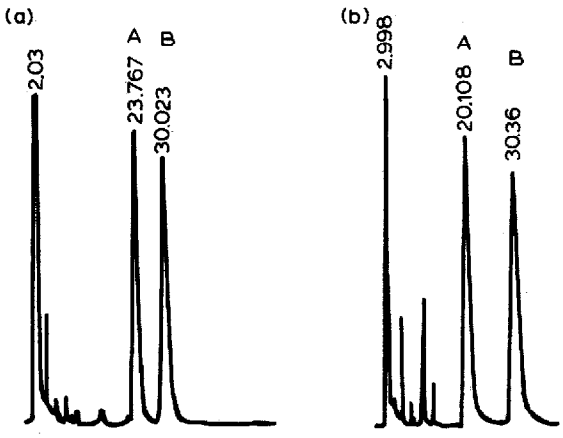

Fig. 1. Separation of NSPI derivatives of (a) racemic amphetamine (peaks A and B) at a flow-rate of 1.0 ml/min, and (b) racemic mexiletine (peaks A and B) at a flow-rate of 1.5 ml/min. Column, silica, 30 × 0.40 cm I.D.; mobile phase, light petroleum–isopropanol (100:3); detection, UV (254 nm). Numbers at peaks indicate retention times in min.

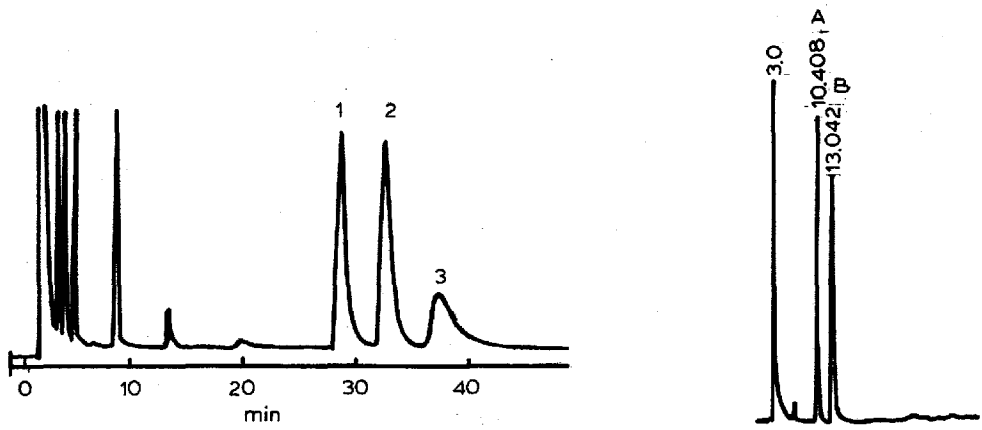

Fig. 2. Separation of the NSPI derivative of (±)-phenylpropanol (peaks 1 and 2). Peak 3 represents the compound formed by heating NSPI at 80°C for 1 h without completely removing the water used for synthesizing NSP-N_3. Mobile phase, light petroleum–isopropanol (100:1); flow-rate, 1.0 ml/min. Column and detection as in Fig. 1.

Fig. 3. Separation of the NSP-N_3 derivative of (±)-amphetamine (peaks A and B). Mobile phase, light petroleum–isopropanol (100:3); flow-rate, 1.0 ml/min. Column and detection as in Fig. 1.

particular, for the optical resolution of alcohols, NSPI can greatly shorten the derivatization time, while NEIC had been reported to resolve racemic 3-O-hexadecylglycerol after derivatization at 80°C for 36 h[3]. One of the reasons may be that the central carbon atom in the isocyanate group of NSPI is more positive and results in greater reactivity than that of NEIC because the nitrogen atom in the proline of NSPI can attract electrons whereas the naphthalene group of NEIC can repel electrons. On the

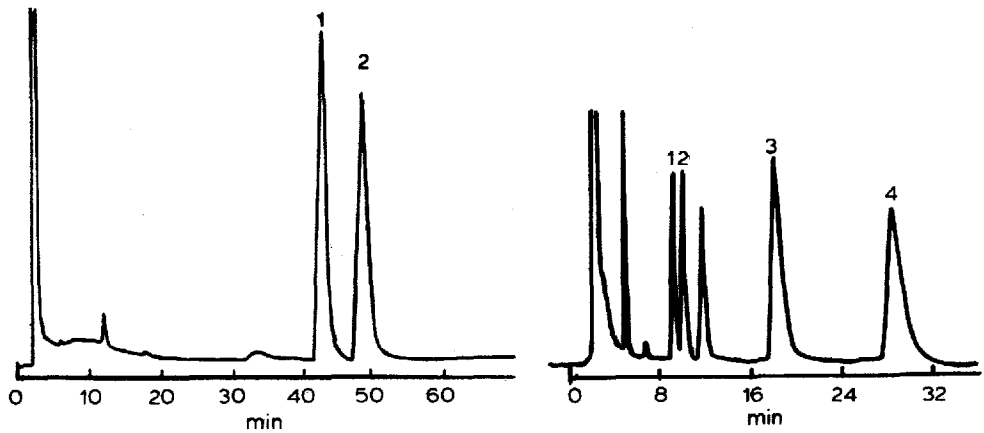

Fig. 4. Separation of the NSP-N_3 derivative of (±)-mexiletine (peaks 1 and 2). Mobile phase, light petroleum–isopropanol (100:1); flow-rate, 1.0 ml/min. Column and detection as in Fig. 1.

Fig. 5. Separation of the (±)-mexiletine derivatives formed with NSP-N_3 (peaks 1 and 2) and NSPI (peaks 3 and 4). Mobile phase, light petroleum–isopropanol (100:3); flow-rate, 1.5 ml/min. Column and detection as in Fig. 1.

other hand, the larger groups connected with the chiral carbon atom of 3-O-hexadecylglycerol may reduce the reactivity of the hydroxyl group with the isocyanate of NEIC.

It is concluded that the indirect separation of amine and alcohol enantiomers with NSPI reagent is a versatile and promising technique.

NSP-N_3 is superior to NSPI owing to shorter retention time and better resolution, as shown in Fig. 5 for the resolution of racemic mexiletine, for instance. The same result can be obtained from amphetamine by comparing Figs. 1a and 3. Amines forms amides with NSP-N_3 and ureas with NSPI. There are only two atoms between the two chiral centres in the amide derivatives, but three atoms in the urea derivatives. According to the principle that in the diastereomeric derivative molecule the distance between the asymmetric carbon atoms of the reagent and the compound to be resolved should be minimized and if possible kept to less than three atoms[9,10] diastereomeric amides should show better separations. In addition, this procedure is more convenient and rapid to operate without heating. Consequently, NSP-N_3 reagent may become a useful chiral resolving agent for amine enantiomers.

REFERENCES

1 G. Pflugmann, H. Spahn and E. Mutschler, *J. Chromatogr.*, 421 (1987) 161.
2 A. Darmon and J. P. Thenot, *J. Chromatogr.*, 374 (1986) 321.
3 P. Michelson, E. Aronsson, G. Odham and B. Åkesson, *J. Chromatogr.*, 350 (1985) 417.
4 W. H. Pirkle and M. S. Hockstra, *J. Org. Chem.*, 39 (1974) 3904.
5 M. Bergman and L. Zervas, *Biochem. Z.*, 203 (1928) 280.
6 S. Reiji, K. Toshio, I. Kazuhiro, F. Yuzo, N. Hiroyuki and T. Nobuchika, *J. Chromatogr.*, 357 (1986) 119.
7 J. Weinstock, *J. Org. Chem.*, 26 (1961) 3511.
8 W. H. Pirkle, G. Mahler and M. H. Hyun, *J. Liq. Chromatogr.*, 9 (1986) 443.
9 B. Feibush and L. Spialter, *J. Chem. Soc., Perkin Trans. 2*, (1971) 106.
10 B. L. Karger, S. Herliczek and R. L. Stern, *Chem. Commun.*, (1969) 625.