Note

Direct stereochemical resolution of enantiomeric amides via thin-layer chromatography on a covalently bonded chiral stationary phase

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In the past few years there has been a dramatic increase in the use of high-performance liquid chromatography (HPLC) chiral stationary phases (CSPs) for the direct stereochemical resolution of enantiomeric compounds. This technique has reached the point where it has been the subject of a number of recent reviews[1-3] and books[4,5]. The use of CSPs in gas–liquid chromatography has also grown and these advances have been discussed in a recent review[6]. The one chromatographic approach which has lagged in the application of CSPs is thin-layer chromatography (TLC).

Most of the initial work in enantioselective TLC using CSPs has centered on the development of phases for the stereochemical resolution of free and derivatized amino acids. The initial chiral resolution of an amino acid by a TLC-CSP was reported by Yuasa *et al.*[7] who resolved D,L-tryptophan on a TLC plate coated with microcrystalline cellulose. Another reported TLC-CSP utilized (+)-tartaric acid-impregnated silica gel plates which were used to resolve stereochemically phenylthiohydantoin amino acids[8]. Plates impregnated with (+)-ascorbic acid were also reported by these authors[8].

An alternative approach to the chiral resolution of free and derivatized amino acids has been chiral ligand-exchange chromatography[9-11]. In this approach reversed-phase TLC plates are impregnated with copper(II) complexed with an enantiomerically pure amino acid derivative. This method has also been used to determine the enantiomeric purity of commercial lots of L-3,4-dihydroxyphenylalanine (L-DOPA)[12] and D-penicillamine[13].

A TLC-CSP with a broader stereoselectivity has been reported by Armstrong and co-workers[14,15], who used β-cyclodextrin-bonded silica. This TLC-CSP has the capacity to resolve a variety of enantiomeric and diastereomeric compounds, including enantiomeric dansyl-amino acids, β-naphthylamide-amino acids and ferrocenyl compounds and the diastereomers quinine and quinidine[14]. In addition, the stereochemical resolutions obtained using the β-cyclodextrin TLC-CSP are comparable to those obtained using the same HPLC-CSP[15].

A class of HPLC-CSPs which also have a broad stereoselectivity are the

Pirkle-type phases. A Pirkle-type TLC-CSP has been reported by Wainer et al.[16], who ionically bonded (R)-N-(3,5-dinitrobenzoyl)phenylglycine (DNPG) to an amino-propyl-silica gel TLC plate. This support was able to stereochemically resolve racemic 2,2,2-trifluoro-1-(9-anthryl)ethanol with a stereochemical separation factor (α) of 1.50. This result was consistent with the α of 1.33 reported for the same enantiomers when resolved on the DNPG HPLC-CSP[17]. However, the TLC-CSP has a high UV background and the analytical applications of this support are currently limited.

An HPLC-CSP similar to DNPG has been described by Oi et al.[18], who reacted (R)-(−)-1-(1-naphthyl)ethyl isocyanate with an aminopropyl HPLC support to produce a naphthylethylurea HPLC-CSP (Fig. 1). A variety of enantiomeric amines and acids were stereochemically resolved on this CSP as the 3,5-dinitrobenzoyl amide (DNB) or 3,5-dinitroanilide (DNAn) derivatives, including 1-phenylethylamine-DNB ($\alpha = 1.85$), valine methyl ester-DNB ($\alpha = 2.02$) and 1-methylphenylacetic acid-DNAn ($\alpha = 2.11$).

In this study, we have reacted the isocyanate used by Oi et al.[18] with a commercially available aminopropyl HPTLC plate to form a naphthylethyl urea TLC-CSP. The resulting TLC-CSP was used to resolve stereochemically the same type of solutes as the naphthylethylurea HPLC-CSP and therefore, should have broad stereoselectivity. Although the napthylethyl chromophore of the present TLC-CSP also has a high UV absorptivity, experimentally we did not observe the detection problems that had been experienced with the DNPG plates. The DNB and DNAn derivatives used in this study could be detected by both short (254 nm) and long (360 nm) UV wavelengths with a limit of detection of 0.5 μg for one of the solutes. The naphthylethylurea TLC-CSP should provide useful alternative in enantioselective TLC.

EXPERIMENTAL

Materials

The (R)-(−)-1-(1-naphthyl)ethyl isocyanate, nitroanilines, acid chlorides and (R)-(+)- and (S)-(−)-α-methylbenzylamines used in this study were purchased from Aldrich (Milwaukee, WI, U.S.A.). The (R)-(−)-ibuprofen was supplied by Upjohn (Kalamazoo, MI, U.S.A.) and the remaining solutes came from the stores of the U.S.
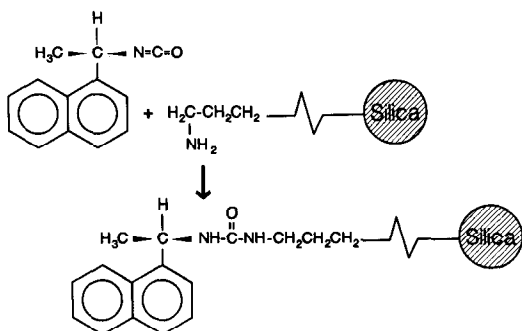

Fig. 1. The synthesis of the naphthylethylurea CSP.

Food & Drug Administration. The aminopropyl-silica gel TLC plates (NH_2, F-254s) were purchased from Alltech (Deerfield, IL, U.S.A.).

Preparation of TLC-CSP plates

A 1-g amount of the isocyanate was dissolved in 100 ml of methylene chloride. An aminopropyl HPTLC plate was soaked in 20 ml of the derivatization solution for 5 min. The plate was removed from the solution and air dried. The TLC-CSP plate was then washed by immersion in methylene chloride (twice) and air dried. The resulting plate was used without further treatment. The amount of isocyanate reagent bound to the plates was calculated from the loss of ultraviolet absorbance of the reagent solution, measured at 281 nm.

General procedure for the synthesis of amide derivatives

The amides derived from amines were synthesized by using the appropriate acid chloride according to a procedure outlined previously[19]. The amides derived from carboxylic acids were synthesized by a previously reported procedure which involved converting the acids to acid chlorides and condensing them with the appropriate nitroaniline[20].

Chromatographic conditions

The TLC-CSP plates were developed using a mobile phase composed of hexane–isopropanol–acetonitrile (20:8:1). The chromatography was carried out in saturated chromatographic tanks, except when the α-methylbenzylamine derivatives were studied.

Detection

The solutes were detected using both short (254 nm) and long (360 nm) UV wavelengths. The lower limit of detection for the 3,5-nitroanilide derivative of solute 1 was 0.5 μg.

Calculations

The R_F values for the solutes were calculated by averaging the results obtained from two separate experiments. The distribution ratios (D) and stereoselectivity values (α) were calculated using the R_F values according to the following equations:

$$D = R_F/(1 - R_F) \tag{1}$$

$$\alpha = D_2/D_1 \tag{2}$$

RESULTS AND DISCUSSION

Preparation of TLC-CSP plates

The TLC plates were prepared by facile room-temperature binding of (R)-($-$)-1-(1-naphthyl)ethyl isocyanate, dissolved in methylene chloride, to the aminopropyl-silanized silica support. The resulting naphthylethylurea chiral stationary phase is identical to the HPLC phase described by Oi et al.[18]. Under the conditions given in the Experimental section, the loading of the plates was 0.41 mmoles per gram of support, or about 86 mg isocyanate reagent per 10 × 10 cm plate.

Chromatographic results

The structures of the compounds used in this study are presented in Fig. 2. Before chromatography on the TLC-CSP plates, the solutes were converted to amides.

A series of racemic α-methylarylacetic acids (solutes 1–5) was converted to the respective 3,5-dinitroanilides and chromatographed on the naphthylethylurea TLC-CSP (Table I). The enantiomeric DNAn derivatives of solute 1 had both the highest R_F values (0.45 and 0.28) and stereoselectivity ($\alpha = 2.10$) of the five solutes studied. The results for the solutes 2–5 were all similar. The R_F values of the least retained enantiomers of the enantiomeric pairs ranged from 0.24 to 0.30; the R_F values for the most retained isomers varied from 0.15 to 0.23. The stereoselectivity ranged from 1.65 to 1.79.

Two chiral amines, solutes 6 and 7, were derivatized with 3,5-dinitrobenzoyl chloride and the resulting amides chromatographed on the naphthylethylurea TLC-CSP (Table I). The R_F values for the enantiomeric DNB derivatives of solute 6 were 0.33 and 0.25 and the observed α was 1.48. For solute 7, the calculated R_F values were 0.37 and 0.31 with $\alpha = 1.31$.
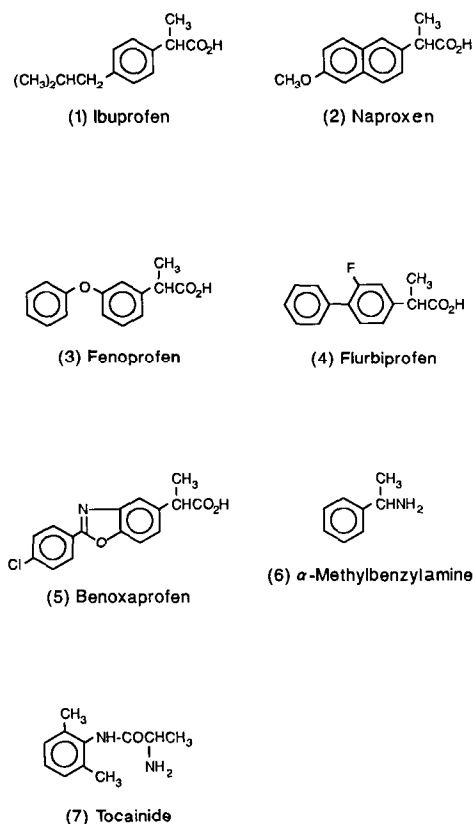

Fig. 2. Structures of the solutes used in this study.

TABLE I

CHROMATOGRAPHIC RESULTS

Compound (see Fig. 2)	Derivative[a]	R_F	α	Order
1	3,5 DNAn-	0.45, 0.28	2.10	R,S
2	3,5 DNAn-	0.24, 0.15	1.79	R,S
3	3,5 DNAn-	0.33, 0.23	1.65	ND[b]
4	3,5 DNAn-	0.33, 0.23	1.65	ND
5	3,5 DNAn-	0.30, 0.20	1.71	ND
6	3,5-DNB-	0.33, 0.25	1.48	S,R
7	3,5-DNB-	0.37, 0.31	1.31	ND

[a] 3,5-DNAn = 3,5-dinitroanilyl; 3,5-DNB = 3,5-dinitrobenzoyl.
[b] ND = not determined.

Stereoselectivity and solute structure

The relative chiral retention order of the enantiomers of solutes 1, 2 and 6 was determined by independent chromatography of at least one of the enantiomers of each pair. For solutes 1 and 2, the enantiomers with the (S)-configuration at the chiral center were more retained than the (R)-isomer while the opposite result was obtained for solute 6; i.e., the (R)-enantiomer was more retained (Table I).

The inversion of the relative chiral retention order of an amide derived from an amine compared to that for an amide derived from a carboxylic acid has been observed on two HPLC-CSPs, the Pirkle-type DNPG-CSP[21] and the cellulose tribenzoate-CSP[22]. The results from these studies suggested that the position of the chiral center relative to the amide moiety is important due the interaction between the amide dipoles of the solute and CSP. This interaction not only plays a role in the formation of the diastereomeric solute–CSP complex but also orients the two molecules within this complex. This orientation ultimately determines the relative stability of the two diastereomeric complexes and, therefore, the relative retentions. The results from this study are consistent with these observations.

The effect of π-acidity on retention and stereoselectivity

The effect of the π-acidity of the aromatic portion of the amide moiety on R_F and α is presented in Table II. For the N-benzoyl derivatives of solute 6, R_F decreases with increasing π-acidity of the benzoyl moiety as represented by the Hammet substituent constants $(\sigma)^{23}$. When the racemic 4-methylbenzoyl derivative $(\sigma = -0.17)$ was chromatographed, the calculated R_F value was 0.59 while for the racemic 4-nitrobenzoyl derivative $(\sigma = +0.78)$ the observed R_F value was 0.49. There was no observed chiral resolution. However, when the racemic 3,5-dinitrobenzoyl derivative was chromatographed, the calculated R_F values were 0.25 for the (R)-enantiomer and 0.33 for (S)-enantiomer with $\alpha = 1.48$.

A similar effect of π-acidity on retention and stereoselectivity was observed for three derivatives of solute 1 (Table II). When the racemic 3-nitroanilide and racemic 4-nitroanilide derivatives of solute 1 were chromatographed on the naphthylethylurea TLC-CSP, the calculated R_F values for the (S)-enantiomers were 0.53 and 0.51, respectively; the R_F values for both (R)-enantiomers were 0.59; the respective

TABLE II

EFFECT OF π-ACIDITY ON R_F AND α

σ = Hammet substituent constants[23].

Ar	σ	R_F	α
$C_6H_5-CH(CH_3)-NH-CO-Ar$			
4-CH$_3$-C$_6$H$_4$	−0.17	0.59	1.0
C$_6$H$_5$	0.00	0.56	· 1.0
4-NO$_2$-C$_6$H$_4$	+0.78	0.49	1.0
3,5-(NO$_2$)$_2$-C$_6$H$_3$	+1.42	0.33(S), 0.25(R)	1.48
$4-(C_4H_9)-C_6H_4-CH(CH_3)-CO-NH-Ar$			
3-NO$_2$-C$_6$H$_4$	+0.71	0.59(R), 0.53(S)	1.28
4-NO$_2$-C$_6$H$_4$	+0.78	0.59(R), 0.51(S)	1.38
3,5-(NO$_2$)$_2$-C$_6$H$_3$	+1.42	0.45(R), 0.28(S)	2.10

stereoselectivities were 1.28 and 1.38. The similarities in the chromatographic results between the two derivatives reflect the fact that the π-acidities of the 3-nitrobenzoyl (σ = +0.71) and 4-nitrobenzoyl (σ = +0.78) moieties are not significantly different. When the 3,5-nitrobenzoyl derivative (σ = +1.41) was chromatographed, there was a significant increase in the retention of both enantiomers, $R_F(S)$ = 0.28 and $R_F(R)$ = 0.45, and in the stereoselectivity, α = 2.10.

These results suggest that one of the key aspects in the chiral recognition mechanism is a π–π interaction between a π-acidic moiety on the solute and the π-basic naphthyl moiety on the CSP. It also indicates that for the best chromatographic results, potential solutes for the naphthylethylurea TLC-CSP which contain an amine or a carboxylic acid moiety should be converted into the corresponding 3,5-dinitrobenzoyl amides or 3,5-dinitroanilides before chromatography.

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