Chiral recognition mechanism study of mandelic acid on whelk O1 column with pre-column derivatization

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ABSTRACT

The mandelic acid was derivatized with four amines and the derivatives were resolved on four types of chiral columns. The results indicated that phenethylamine derivative was resolved well on all the four columns and the Whelk O1 column had the best resolution. The resolution of phenethylamine derivative on Whelk O1 column was then validated before the mechanism study. The influence of temperature on chiral recognition of phenethylamine derivative had been investigated on Whelk O1 column. By analyzing thermodynamic data of chiral separation process, the mechanism of chiral separation was tentatively investigated. The results suggested that the separation was mainly controlled by steric hindrance, and it was an enthalpy-driven course. Chromatographic model was employed for enantionmeric separation of mandelic acid derivatives on Whelk O1 column using hexane/ethanol mobile phase.

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INTRODUCTION

Mandelic acid, α-hydroxyphenyl acetic acid, is intensively investigated in recent years as one of the most useful fine chemicals and pharmaceutical intermediates[1]. Optical mandelic acid is a very useful acidic resolving agents[2]. R-mandelic acid is used in the side-chain modification of cefadole, and S-mandelic acid is the precursor of S-oxybutynin. With the development of chiral drugs, the need of single enantiomer of mandelic acid increases very fast. But the approaches to produce enantiopure mandelic acid actually produce only enantiomer-rich mandelic acid[3-6]. So it is important to effectively resolve and precisely determine the optical purity of mandelic acid in the asym-

metric synthesis procedure[7].

The traditional method to determine optical purity is carried out by a polarimeter method. But its precision is often affected by other optical impurities. The chromatographic method develops very fast in the past years, such as chiral mobile phase additive method[8], capillary electrophoresis[9] and gas chromatography method[10] and chiral stationary phase (CSP) method[11]. But there are still flaws such as low separation factor, long analysis time, unsatisfactory peak shape, poor quantitation capacity and so on.

Due to its hydroxyls and unsuitable stereochemistry, mandelic acid enantiomers cannot be directly resolved by most of the chromatographic columns. In the following contents, four derivatization agents were cho-

sen to derivatize racemic mandelic acid including aniline, phenethylamine, p-methyl aniline, and methyl naphthylamine. The derivatives were subsequently resolved by CSPs with mobile phases of different iso-propanol or ethanol contents. The best way to resolve mandelic acid was then validated. Influence of temperature on resolution of phenethylamine derivative on Whelk O1 column was also investigated. Then the chiral separation mechanism was tentatively analyzed by thermodynamic data obtained. It deserves to be referred in the similar mechanism study and also can be a guideline for future analysis programs.

EXPERIMENTAL

Chemicals and reagents

Mandelic acid, aniline, phenethylamine, p-methylaniline, methyl naphtylamine, N, N'-Dicyclohexylcarbodiimide (DCC), methylene chloride are of analytical grade. Hexane, isopropanol, anhydrous ethanol and methanol are of chromatographic grade. Other chemicals are of analytical grade. All the chemicals and reagents are purchased from Kelong Chemical Reagent Factory.

Instruments and equipments

Analysis was carried out on a Shimadzu series liquid chromatography system, equipped with LC-20AT pump and SPD-M20A photodiode array detector (both from Shimadzu, Kyoto, Japan), JASCO (Japan) model OR-2090 optical rotation detector, and an Automatic Science (Tianjin, China) HCT-360 LC column cooler/heater. Chromatographic parameters such as peak area, retention time, and theoretical plates were calculated using the Class-VP workstation (Shimadzu, Kyoto, Japan).

Derivatization procedures

Mandelic acid (0.15g, 1mmol) and DCC (0.41g, 2mmol) were added in a flask charged with 60.0mL of anhydrous methylene chloride. The mixture was stirred at room temperature for 5min. Then 1 mmol of specific amine was added in the system. The reaction mixture was kept stiring at 303.15K for 3h. The reaction was given below in Scheme 1. The undissolved impurities were removed by filtration. The filtrate was then washed by diluted hydrochloric acid, aqueous NaOH solution and distilled water in sequence. The organic phase was removed under vacuum after drying by anhydrous $NaSO_4$. Finally the product was obtained as white or yellowish solid[12].

$R \quad C_6H_5-\quad C_6H_5CH_2CH_2-\text{p-}CH_3\text{-}C_6H_5\text{-}\alpha\text{-}CH_3\text{-}C_{10}H_6\text{-} \quad 1a\text{-}d$
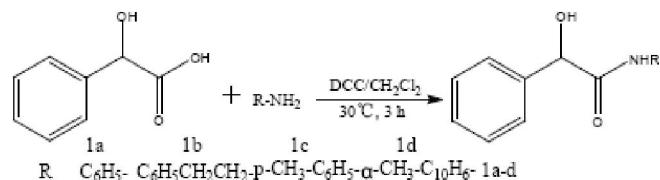
Scheme 1 : The derivatizing reaction of mandelic acid

Chromatographic conditions

Chiralcel OD-H (250×4.6mm; particle size 5μm) (Daicel, Japan), DNB-PG (250×4.6mm; particle size 5μm), Whelk-O1 (250×4.6mm; particle size 5μm) (Regis Technologies, USA) and Kromasil CHI-DMB (250×4.6mm; particle size 5μm) (Akzo Nobel, Sweden) were used for the separation. Mobile phase was consisted of n-hexane/isopropanol or ethanol (85/15 or other v/v) and the column temperature was 293.15K. The flow rate was 1.0mL/min and the detection wavelength was set at 254nm. Void times were determined using ethanol as a marker. The injection volume was about 5μL. The sample solution was prepared by dissolving the sample in methanol at 100μg/mL.

RESULTS AND DISCUSSION

Separation results of different columns

The optimized mobile phases for CHI-DMB and DNB-Leucine was hexane/isopropanol (90/10, v/v), and for OD-H and Whelk O1 was hexane/ethanol (85/15, v/v). The derivatives were resolved on the columns mentioned above under the optimized mobile phases and TABLE 1 showed the results.

As known to all, the larger the separation factor (α) is, the better the resolution will be. Thus the Whelk O1 column had the best resolution effect. The chromatograms were given in Figure 1. And the phenethylamine derivative was resolved well on all the columns. The resolution of phenethylamine derivative on Whelk O1 column was validated referred to the parameters including precision, accuracy, linearity, limit of detection (LOD), limit of quantification (LOQ) and robustness. Thereby, the best way to analyze mandelic acid in this

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TABLE 1 : Comparison of resolution for four derivatives of mandelic acid on four columns

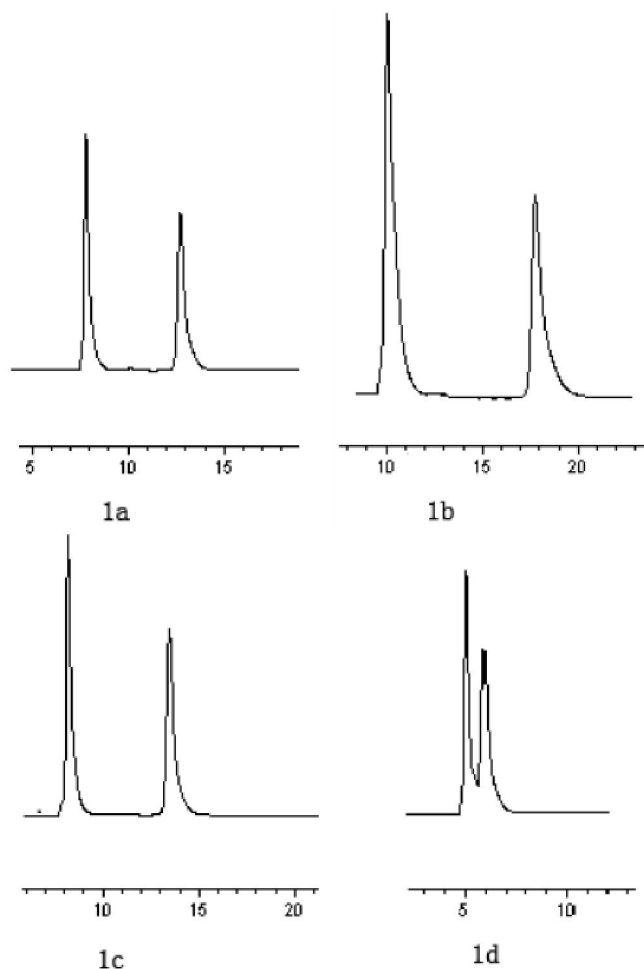| Derivatives		1a	1b	1c	1d
CHI-DMB	t_{R1} /(min)	5.973	4.661	5.611	7.787
	t_{R2} /(min)	6.421	5.312	6.005	8.693
	α	1.159	1.430	1.160	1.195
DNB-Leu	t_{R1} /(min)	10.923	14.837	11.552	14.400
	t_{R2} /(min)	12.000	17.568	12.363	14.400
	α	1.139	1.234	1.097	1.000
OD-H	t_{R1} /(min)	7.349	6.421	7.605	9.856
	t_{R2} /(min)	9.024	8.032	8.043	10.507
	α	1.399	1.492	1.098	1.097
Whelk O1	t_{R1} /(min)	7.776	8.256	8.139	5.781
	t_{R2} /(min)	12.683	14.837	13.429	6.080
	α	2.060	2.288	2.060	1.114

Figure 1 : Chromatograms of the four derivatives resolved on Whelk O1 column

research was established: firstly, the mandelic acid was derivatized with phenethylamine; then it was analyzed

TABLE 2 : Resolution results of phenethylamine derivative on Whelk O1 CSP

T /(K)	t_{R1}/ (min)	t_{R2} /(min)	α
283.15	9.845	18.987	2.365
288.15	9.504	17.867	2.316
293.15	9.227	16.949	2.270
298.15	9.088	16.416	2.234
303.15	8.565	15.029	2.193
308.15	7.925	13.429	2.152
313.15	7.339	12.032	2.120

with Whelk O1 CSP at 293.15K under 254nm. Hexane/ethanol (85/15, v/v) was used as the mobile phase.

The influence of temperature on the resolution

Temperature is an important factor affecting chiral recognition process. The influence of column temperature on selectivity and resolution of the derivatives of mandelic acid enantiomers were studied in the range 283.15-313.15K. Since all of the derivatives had the same trend, the resolution results of phenethylamine derivative were provided as an example in TABLE 2. With the increase of temperature, the retention decreased and the separation factors decreased. These results could be attributed to the fact that the analytes on molecular level had lower adsorption as temperature increased and therefore migrated faster through the column.

In the enantiomer separation process, the relationship between capacity factor of solutes(k') and column temperature together with the relationship between separation factor(α) and column temperature can be inter-

TABLE 3 : Regression equations of different derivatives

Derivatives		Correlation between ln k' and T			Correlation between ln α and T		
	K	a/(K)	b/(K)	r^2	a'/(K)	b'/(K)	r2
1a	k1'	936.0	2.854	0.970	295.5	0.268	0.996
	k2'	1231	3.112	0.982			
1b	k1'	1266	3.679	0.926	340.3	0.340	0.995
	k2'	1607	4.019	0.948			
1c	k1'	977.9	2.924	0.938	305.7	0.302	0.990
	k2'	1283	3.266	0.957			
1d	k1'	1123	4.046	0.962	125.3	0.314	0.980
	k2'	1248	4.360	0.968			

related by Gibbs-Holmholtz equation(1) and Van't Hoff equation(2), respectively.

$$\ln k' = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln \varphi \qquad (1)$$

$$\ln \alpha = -\frac{\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \qquad (2)$$

TABLE 4 : Thermodynamic data of mandelic derivatives on Whelk O1

Derivatives	ΔH_1 /(kJ/ mol)	ΔH_2 (kJ/ mol)	$\Delta(\Delta H)$ (kJ/ mol)	$\Delta(\Delta S)$ /(kJ/mol /K)	$\Delta(\Delta G)$ /(kJ/ mol)	T_β /(K)
1a	-7.781	-10.234	-2.456	-2.228	-1.792	1102.334
1b	-10.525	-13.360	-2.829	-2.827	-1.986	1000.707
1c	-8.130	-10.666	-2.541	-2.511	-1.792	1011.947
1d	-9.336	-10.375	-1.041	-2.611	-0.263	398.698

Where k' is capacity factor of solutes, ΔH and ΔS are the enthalpy difference and entropy difference of solutes during the retention course, R is gas constant, T is temperature(K), φ is phase ratio, $\Delta(\Delta H)$ is the difference of enthalpy difference between enantiomers and it reflects the difference of interactions between enantiomers and CSP, $\Delta(\Delta H)$ is the difference of entropy difference between enantiomers and it reflects the difference of enantiomers in the two phases. Using $\ln K'$ or $\ln \alpha$ as ordinate, and $\frac{1}{T}$ as abscissa, the corresponding Van't Hoff and Gibbs-Holmholtz curves were obtained. Their regression equations were given in TABLE 3, and the further calculation results of thermodynamic data were given in TABLE 4. Where in TABLE 3, α is equal to $-\Delta H/R$, b is equal to $-\Delta S/R - \ln \varphi$, α' is equal to $-\Delta(\Delta H)/R$, b' is equal to $-\Delta(\Delta S)/R$.

TABLE 3 showed that $\ln \alpha$, $\ln k_1'$ and $\ln k_2'$ had a linear relationship with $\frac{1}{T}$. $\ln k_1'$ and $\ln k_2'$ decreased with the increase of temperature. The Van't Hoff curves were nearly linear. This indicated that the retention mechanism of the enantiomers did not change with the temperature, and the chiral separation was controlled by adsorption reaction heat. ΔHs were negative during

the retention course of the enantiomers judged by the Van't Hoff curves. $\Delta(\Delta H)$ and $\Delta(\Delta S)$ were also negative. This indicated the interactions between solutes and CSP were exothermal, and thus the chiral separation was an enthalpy-driven course. Furthermore, the separation was a minus entropy change course. This was detrimental for the separation, and it must be compensated by enthalpy released in the chiral recognition course between enantiomers and CSP. Only by this way can derivatives of mandelic acid be resolved.

The investigation of separation mechanism

For Whelk O1CSP, steric hindrance adjacent to the chiral carbon played an important role in the resolution. Due to the extension of chain, the steric hindrance of phenethylamine derivative was smaller than that of aniline derivative. p-Methylaniline had nearly the same steric hindrance with aniline, and the steric hindrance of
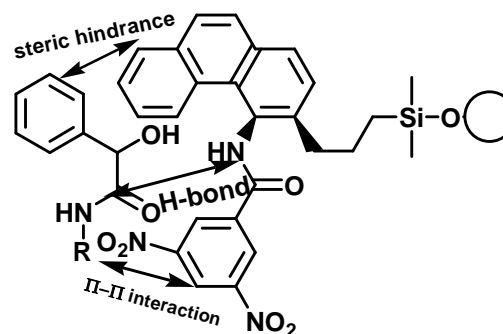

Figure 2 : The interaction sites between derivatives of mandelic acid and CSP

methyl naphtylamine derivative was the largest. The smaller the steric hindrance was, the longer the retention time was, and the better the resolution was. In addition, the aromatic ring of p-methylaniline derivative was born with a π-basic methyl which made the interaction with the π-acidic aromatic ring of the CSP stronger. So the retention time was longer than that of aniline derivative, but with no significant separation influence.

The chiral selector of Whelk O1 CSP bears both π-acidic and π-basic groups. Its chiral recognition is in accordance with three-point principle. The interactions occur between the enantiomers and the CSP mainly include: (1) π-π interaction between solutes and π-acceptor and π-donor of aromatic ring of the CSP; (2) H-bond interaction between acidic proton, hydroxyl and amino of solutes and parahelium and carbonyl of CSP;

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(3) dipole-dipole interaction; (4) steric effect of bulky groups adjacent to the chiral centre of CSP[13, 14]. In this research, three interaction sites between derivatives of mandelic acid and CSP were given in Figure 2.

It can be seen from Figure 2 that different substitute groups resulted in different steric hindrance adjacent to chiral carbon. Then it affected the H-bond between enantiomers and CSP. This in return made the enantiomers had different interactions with CSP. Finally, the enantiomers were separated. $\Delta(\Delta H)$ s were quite small as can be seen from TABLE 4. These differences were not enough to make the separation feasible. The enantiomer separation may result from the tiny differences arose from steric hindrance. These tiny differences may attribute to the H-bond, π-π, dipole interactions, Van der Waals', and dispersion forces. In addition, the absolute value of $\Delta(\Delta H)$ of phenethylamine derivative was the largest of the four, and its resolution was the best. This verified the conclusion of steric hindrance effect.

CONCLUSION

The resolution of the four derivatives of mandelic acid was carried out on CHI-DMB, DNB-Leucine, OD-H and Whelk O1. The resolution on Whelk O1 was the best of the four. Three of the four derivatives were baseline separated with quite good results. The separation factor were all larger than 2. Comparing the steric hindrances adjacent to chiral carbon of the four derivatives, it can be concluded that steric hindrance is one of the most important influencing factors for the separation factor. For Whelk O1, the influence of temperature on the separation was investigated under the mobile phase hexane/ethanol (85/15). The lower the temperature was, the larger the separation factor was. But if the temperature was too low, the retention time was too long, and it was detrimental for fast analysis. Finally, by calculating the thermodynamic parameters of the chiral recognition process, the resolution mechanism was investigated tentatively. The chiral recognition was an enthalpy- driven course. This mechanism study will be helpful in the future chiral analysis.

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