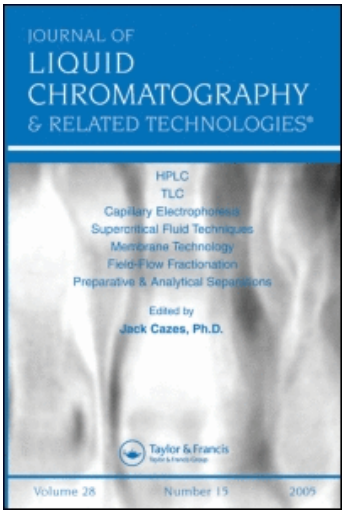


**Journal of Liquid Chromatography & Related Technologies**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

# Optical Resolution of Some Mandelic Derivatives on a Chemically Bonded Cyclodextrin Stationary Phase

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**To cite this Article** Fujimura, Kazumi , Kitagawa, Masahi , Takayanagi, Hiroaki and Ando, Teiichi(1986) 'Optical Resolution of Some Mandelic Derivatives on a Chemically Bonded Cyclodextrin Stationary Phase', Journal of Liquid Chromatography & Related Technologies, 9: 2, 607 — 620
**To link to this Article: DOI:** 10.1080/01483918608076656
**URL:** http://dx.doi.org/10.1080/01483918608076656

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(2&3), 607-620 (1986)

# OPTICAL RESOLUTION OF SOME MANDELIC DERIVATIVES ON A CHEMICALLY BONDED CYCLODEXTRIN STATIONARY PHASE

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## ABSTRACT

Preparation and use of a new type of cyclodextrin-bonded stationary phase are described. Resolution of racemic mandelates and their analogues has been achieved by using this phase. The chiral recognition has been explained in terms of a three-point attachment model. In addition to the inclusion of the aromatic ring of the sample into the cavity of cyclodextrin, enantioselective interaction occurs at two other points; hydrogen bonding of the two polar substituents on the asymmetric carbon atom with secondary hydroxyl groups on the rim of the wider opening face of the cyclodextrin molecule. The effect of the type and position of substituents and other factors responsible for the resolution is discussed.

## INTRODUCTION

Optical resolution of racemates is of particular significance in synthetic, pharmaceutical, and biological chemistry. Among a



 0148-3919/86/0902-0607$3.50/0

variety of separation methods, chromatographic techniques are most commonly used because they can offer not only analytical information but also an actual separation of enantiomers within a limited analysis time.

Several chromatographic techniques have been proposed for direct resolution of racemates. As the retention mechanism, hydrogen bonding and ligand-exchange are the most typical in direct resolution using a chiral stationary or mobile phase. Optical resolution by solvent-solute chiral interaction in gas chromatography (GC) and by molecule-to-molecule diastereomeric interaction in liquid chromatography (LC) are both based on the former, whereas the latter has been employed exclusively in LC. Although GC using microbore capillary columns offers a high column efficiency, high-performance liquid chromatography (HPLC) is thought to be more suitable for the resolution of less volatile samples and for semi-preparative separation. However, LC resolution of racemates based on either of the two mechanisms still has some problems; addition of chiral species or their metal complexes can cause a serious contamination of effluents. Moreover, the design or preparation of such species is difficult in some cases.



Cyclodextrins (CD's) are now of importance in chemistry and industry owing to their enzyme-like behavior. Since a CD molecule is optically active in itself and the formation of inclusion complexes is a dynamic process, CD's can be used for the chromatographic resolution of racemates. There are some papers describing the use of α- and β-CD-bonded polymer gels for the optical resolution of mandelic acid derivatives or other compounds in column chromatography [1-4]. Addition of CD to the aqueous mobile phase as a chiral eluent was also reported [5, 6]. Although the results obtained in these systems reveal an essential advantage of the use of CD, problems still remain. Soft polymer gels are not suitable for a high-pressure column, leading to a prolonged analysis time and low efficiency. Addition of CD's to the aqueous mobile phase often has a limitation because of the low solubility of CD's, especially that of β-CD [7]. From the practical point of view, CD-bonded rigid materials such as microparticulate silica gels are

indispensable for constructing an effective chromatographic system.

Recently we prepared some chemically bonded CD stationary phases for the separation of aromatic compounds [8, 9], where CD molecules were bonded to silica gels via nitrogen-containing alkyl groups. The secondary amino groups in the spacer chain as well as the residual amino groups on the silica surface were found to behave as anion-exchangers in a low pH region, which sometimes had an interfering effect for the separation by inclusion complex formation. Different types of CD-bonded stationary phases were also reported, but they either have still other functional groups [10, 11], or have structures not known yet, even though a high selectivity was claimed [12, 13]. It is of importance, therefore, to obtain a new CD-bonded phase which brings about no additional retention mechanism except for inclusion complex formation in order to investigate the nature of the chiral recognition by CD molecules. This paper describes the preparation and the retention characteristics of a CD carbamate-bonded stationary phase. Mandelic acids and related compounds were selected as the samples. The effect of the type and position of substituents on resolution was examined and the dependence of the resolution on the sample structure is discussed based on a three-point attachment model. Since CD's are known to prefer aqueous media in inclusion complex formation, a hydroorganic mobile phase was exclusively used. As the organic modifier of the mobile phase was mainly used methanol, as described previously.



## EXPERIMENTAL

### Apparatus.

The HPLC system was composed of a Waters Model 6000A solvent delivery pump (Waters Associates, Milford, MA), a Rheodyne Model 7120 sample injector with a 20 μL sample loop (Rheodyne Inc., Berkeley, CA), and a Jasco UVIDEC-100-II variable wavelength UV detector (Jasco, Tokyo, Japan). The flow rate was 0.7 mL/min and

the detector was operated at 254 nm. Sample size was usually 1 μL.

Modified silica gels were packed in stainless steel columns (200 x 4.0 mm i.d.) by the balanced-viscosity method using a Chemco Model 24 slurry packing apparatus (Chemco Scientific Co. Ltd., Osaka Japan) at ca 450 kg/cm$^2$.

Infrared (IR) spectra of the reaction products during the preparation of the CD-bonded phase were obtained on a Shimadzu IR-400 infrared spectrometer (Shimadzu Corp., Kyoto, Japan) using a liquid sample cell.

## Chemicals and Materials.



Silica gel used was Cosmosil 5SL, totally porous spherical silica, of 5 μm mean particle diameter and 330 m$^2$/g surface area, purchased from Nakarai Chemicals (Kyoto, Japan). α- and β-Cyclodextrins were also purchased from Nakarai Chemicals. Silylating agents, trimethoxysilylpropyldiethylenetriamine and 3-isocyanatopropyltriethoxysilane (Petrarch Systems Inc., Bristol, PA), were purified by distillation before use.

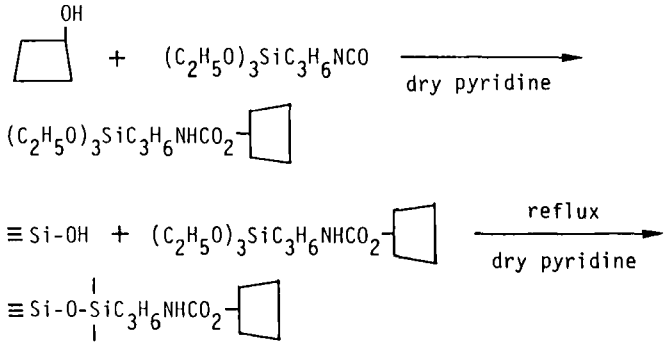
D- and L-Mandelic acids and other chemicals were of the highest quality available and were purchased from various suppliers. Some optically active or racemic samples were prepared in our laboratory according to the literature method [14]. Samples were dissolved in methanol or aqueous methanol so that solutions of ca 1 mg/mL were obtained.

HPLC grade methanol (Nakarai Chemicals) was used as received. Deionized water was further purified on a Milli-Q Water Purification System (Millipore Corp., MA). Aqueous methanol was filtered through a membrane filter of 0.7 μm pore size, and was degassed by ultrasonic vibration under vacuum.

## Preparation of Cyclodextrin Stationary Phases.

CD-carbamate stationary phases were prepared as follows. α-CD (9.7 g) or β-CD (11.4 g), dried at 100 °C in vacuo for 4 h, was dissolved in dry pyridine (100 mL). Then 3-isocyanatopropyltri-

ethoxysilane (6.6 g) was added dropwise into the solution under nitrogen atmosphere with stirring. The solution was kept at ca 60 °C until the absorption at 2200-2300 $cm^{-1}$ (N=C=O) completely disappeared. Formation of carbonyl group was also monitored by IR spectrometry (1700 $cm^{-1}$). To this solution silica gel (5 g), which had been dried at 130 °C in vacuo for 6 h , was added with stirring. The reaction mixture was refluxed for 20 h. After cooling, the modified silica was filtered, washed successively with pyridine, acetone, methanol and water, and then dried at 50 °C in vacuo. Reaction schemes are as follows:




Triamine-CD stationary phase was prepared similarly as Diamine-CD stationary phase [8]. Trimethoxysilylpropyldiethylenetriamine was used as the silylating agent.

## RESULTS AND DISCUSSION

### Characterization of CD-bonded Silica Gels.

The amounts of bonded CD's were calculated from the results of CH and N elemental analyses. The values for α- and β-CD carbamate- and Triamine-β-CD-bonded phases were 109, 127, and 53.8 μmol/g packing, respectively. This suggests that CD-carbamate-bonded phases are superior to the amine-bonded one in two ways; no addi-

tional retention is expected and a much more (about twofold) amount of CD was bonded. As for the chromatographic selectivity of these three bonded phases, only the β-CD carbamate-bonded phase gave satisfactory resolution of racemates. The discussions below are based on the data obtained on the β-CD carbamate-bonded stationary phase.

## Effect of Organic Modifier in Mobile Phase on Retention and Resolution.

The retention times of samples were all reduced with an increase of methanol content in the mobile phase. The effects of the mobile phase composition on capacity factors (k's), separation factor (α) and peak resolution ($R_s$) of methyl DL-mandelate are illustrated in Figure 1. The $R_s$ value had the maximum at ca 5 % methanol, indicating that methanol is also responsible for improving peak shapes. When the methanol content was higher than 20 %, resolution of racemates could not be achieved except for tropic acid derivatives, because retention of samples were very weak.

Similar retention behavior was observed when ethanol was used as the modifier. There was no difference in retention nor in selectivity between methanol and ethanol at the same solvent strength. This suggests that the addition of an organic modifier to the mobile phase does not improve the selectivity for racemates, though it may be effective for obtaining proper retention times and sharp peaks. This effect is probably due to the fact that the formation of inclusion complexes is more or less restricted in the presence of an organic modifier.

Resolution of racemates of free mandelic or other carboxylic acids could not be achieved when a neutral aqueous or hydroorganic mobile phase was used. These acids showed much smaller retention than their esters, indicating that these compounds are ionized in such mobile phases. Adjustment of the pH of the mobile phase was somewhat difficult because the bonded phase is unstable in a low pH region.

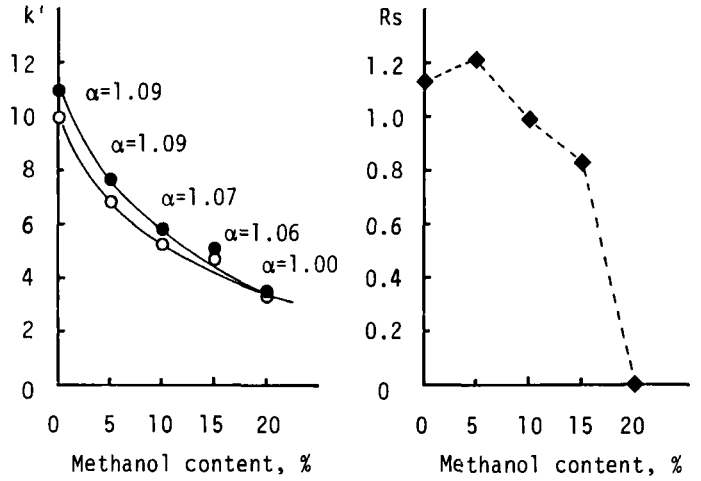



FIGURE 1. Effect of methanol content in mobile phase on capacity factor, separation factor and peak resolution of methyl D-mandelate (○) and methyl L-mandelate (●). Column; β-CD carbamate, 200 x 4 mm i.d. Eluent; methanol/water (5/95). Flow rate; 0.7 mL/min.



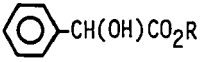
Resolution of Mandelates.

Some esters of mandelic acid were resolved on the CD-bonded phase using 5 % methanol as the mobile phase. Table 1 lists the capacity factors and separation factors. The data in the table reveal that an increase in the carbon number of the ester group brings about a decrease in selectivity, i.e., longer alkyl chains inhibit the chiral recognition by CD molecules. Isopentyl or benzyl mandelates were eluted from the column by using a methanol-rich mobile phase, but the optical resolution of these solutes could not be achieved. This may be attributed to the sorption of the ester group into the cavity of the CD molecule as well as onto the spacer chain.

Chromatograms of methyl, ethyl and propyl mandelates are shown in Figures 2, 3(a) and 3(b), respectively. The elution order of the racemates were the same as had been reported previously [3].

TABLE 1

Capacity Factors and Separation Factors for Enantiomers of Mandelic Acid Derivatives.



| Compound -R | k' D- | k' L- | α |
|---|---|---|---|
| -H | 0.83 | 0.83 | 1.00 |
| $-CH_3$ | 6.94 | 7.54 | 1.09 |
| $-CH_2CH_3$ | 8.99 | 9.61 | 1.07 |
| $-CH_2CH_2CH_3$ | 20.0 | 20.8 | 1.04 |
| $-CH_2CH_2CH(CH_3)_2$ | -[a] | - | |
| $-CH_2-C_6H_5$ | - | - | |

Column; β-CD carbamate, 200 x 4 mm i.d. Eluent; methanol/water (5/95). Flow rate; 0.7 mL/min.
a) Not eluted.



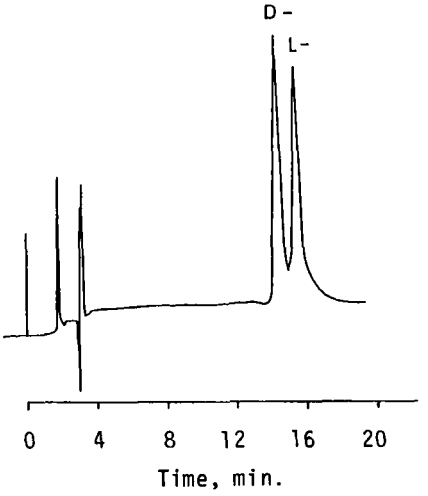



FIGURE 2. Separation of methyl DL-mandelate.
Chromatographic conditions are the same as in FIGURE 1.

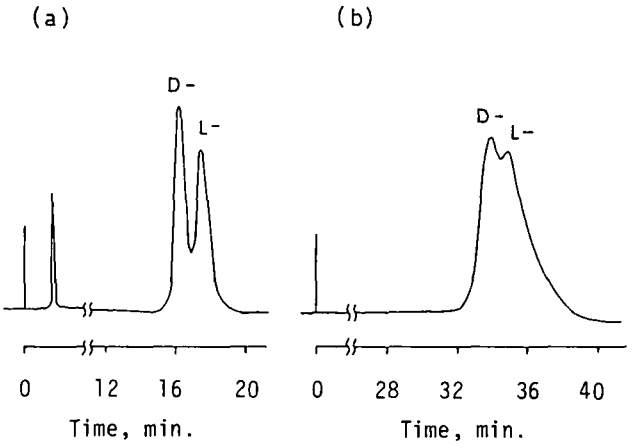



FIGURE 3. Chromatograms of ehtyl DL-mandelate (a) and propyl DL-mandelate (b).
Chromatographic conditions are the same as in FIGURE 1.



Effect of Substituent on Benzene Ring.

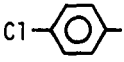
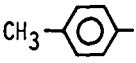
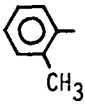
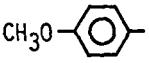
When a sample is retained on the CD-bonded phase, the benzene ring is probably sorbed into the cavity of the bonded CD molecule. To elucidate the possible effects of the stability of inclusion complexes on retention and resolution, the effect of the substituent on the benzene ring was examined. The results are given in Table 2. All substituents examined showed a similar steric effect on retention, which is in accordance with the general expectation that the stability of inclusion complex of disubstituted benzene derivatives follows the order of ortho- < para-isomer. It is to be noted that a para-substituent, though it gives a larger retention, is not effective for improving the enantioselectivity. The elution order of each pair of enantiomers was assumed to be the same as that of mandelic esters.

The fact that neither the alkyl chain of the ester group nor the substituent on the benzene ring of mandelic esters is effective for improving chiral recognition indicates that the enantioselec-

TABLE 2

Effect of Substituent on Benzene Ring on Capacity Factors and Separation Factors for Enantiomers of Methyl Mandelates.

$Ar\text{-}CH(OH)CO_2CH_3$

| Compound Ar- | k' D- | k' L- | α |
|---|---|---|---|
|  | 6.94 | 7.54 | 1.09 |
|  | 22.0 | 23.3 | 1.06 |
|  | 7.90 | 7.96 | 1.00 |
|  | 17.2 | 18.4 | 1.07 |
|  | 4.96 | 4.96 | 1.00 |
|  | 13.7 | 14.4 | 1.06 |

Chromatographic conditions are the same as in Table 1.

tivity cannot be explained by the inclusion of the benzene ring into the cavity of a CD molecule alone. There should, therefore, be other interactions between the sample mandelates and bonded CD molecule which are directly related to chiral recognition. Since the internal surface of a CD molecule has only one type of strongly interacting functional group, secondary hydroxyl, hydrogen bonding between these hydroxyl groups and the polar substituents on the

asymmetric carbon atom of mandelic acid is considered to be responsible for the enantioselectivity. In view of the symmetrical structure of a CD molecule, both of the two substituents of mandelates, carbonyl and hydroxyl, must be concerned with the chiral hydrogen bonding. Thus, it is most probable that the chiral recognition for mandelates is based on a three-point attachment model; inclusion of the benzene ring and two-point hydrogen bonding.

Resolution of Related Compounds.

To test the validity of the three-point attachment model described above, the effect of the type of substituent on the asymmetric carbon atom of mandelic type acid on resolution was examined. The results are summarized in Table 3.

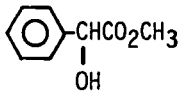
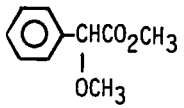
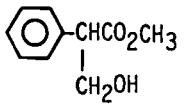
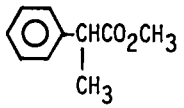
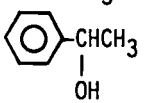
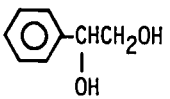
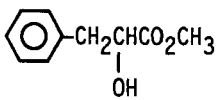
Concerning the substituent replaced for the hydroxyl group, methoxy-substituted (methyl O-methylmandelate) or hydroxymethyl-substituted (methyl tropate) derivatives were resolved, whereas methyl-substituted (methyl 2-phenylpropionate) or amino-substituted (methyl phenylglycinate) ones were not. This suggests that the presence of an oxygen atom is necessary for the formation of a chiroselective hydrogen bonding though the requirements for the distance between the carbon and oxygen atoms are seemingly not very severe.

Similarly, the presence or absence of an ester carbonyl group has been found to have a significant effect: no selectivity was observed for hydroxymethyl-substituted (styrene glycol) or methyl-substituted (1-phenylethanol) derivatives.

Substitution of the phenyl group or the α-hydrogen atom of mandelic ester also affects the selectivity. Methyl 3-phenyllactate, which has an additional methylene group between the phenyl group and the asymmetric carbon atom, was not resolved, suggesting that the chiral carbon the atom must be adjacent to the benzene ring. Methyl 2-hydroxy-2-phenylpropionate was not resolved, ei-

TABLE 3

Capacity Factors and Separation Factors of Enantiomers of Some Related Compounds.

| Compound | k' | | α |
|---|---|---|---|
| | D- | L- | |
|  | 6.94 | 7.54 | 1.09 |
|  | 16.7 | 17.8 | 1.06 |
|  | 17.4 | 19.1 | 1.10 |
|  | 13.9 | 13.9 | 1.00 |
|  | 11.1 | 11.1 | 1.00 |
|  | 6.31 | 6.31 | 1.00 |
|  | 15.0 | 15.0 | 1.00 |

Chromatographic conditions are the same as in Table 1.

ther. Probably, the methyl substituent on the asymmetric carbon atom inhibits the formation of inclusion complex in this case.

From the discussions above, it may be concluded that the chiral recognition by bonded CD is based on a three-point attachment model. Investigation on the retention behavior of other aromatic compounds is expected to give more information on the advantage of CD-bonded phases.

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