On-line HPLC−Exciton CD Analysis Using a Chiral Benzoyl Agent, (*S*)-TBMB Carboxylic Acid: A Promising Approach toward Selective Identification of Enantiomeric Diols and Diamines

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The circular dichroism monitoring HPLC [on-line HPLC−CD] system allows selective identification of those asymmetric compounds in a crude matrix that give Cotton effects in the UV wavelength region.[1] This convenient method is, however, invalid for the analysis of nonchromophoric compounds such as alcohols and amines. For this purpose, either precolumn or postcolumn derivatization with a pertinent CD chromophoric agent must be used. According to the well-known "dibenzoate chirality method" of Nakanishi and Harada,[2] strong exciton CD curves result from benzoylation of asymmetric diols or diamines.[2−10] The bisignate CD peaks reflect the absolute geometry between the two functional groups and appear at a particular position characteristic of the benzoyl chromophore. These CD phenomena established by the exciton CD chirality rule[2] suggest that the on-line HPLC−CD analysis using precolumn benzoylation will become a convenient method not only for the selective detection of asymmetric compounds bearing vicinal diols and diamines but also for the determination of their absolute configuration. In the present study, we apply a chiral benzoyl agent to this method, called "*on-line HPLC-exciton CD analysis*", to propose a promising method for the selective identification of enantiomeric diols and diamines.

(*S*)-TBMB carboxylic acid [(*S*)-2-*tert*-butyl-2-methyl-1,3-benzodioxole-4-carboxylic acid][11,12] is a new type of chiral benzoic acid with a 1,3-benzodioxole skeleton. Its strong fluorescence [excitation 310 nm, emission 380 nm] and high potential to separate enantiomeric alcohols and amines have been conveniently applied to the enantiomer analyses of acyl glycerides, carbohydrates, and amino acids based on fluorescence-monitoring HPLC.[13] This agent is also designed to function as a benzoate CD chromophore to determine the absolute configurations of asymmetric alcohols and amines based on the exciton CD chirality method.[14] In the present on-line HPLC−exciton CD analysis, all of these functions of (*S*)-TBMB carboxylic acid cooperate to provide a simultaneous method to separate and identify enantiomeric diols or diamines in both selective and sensitive manners.

Table 1. On-line HPLC−Exciton CD Data of 1,2-Diols and 1,2-Diamines as Their Di-(*S*)-TBMB Carbonyl Derivatives

diols or diamines	HPLC conditions[a]	k' [b]	peak signs (215/225 nm)
cyclohexane diols	(A)		
trans-(1*S*,2*S*)-**1**		0.98	negative/positive
(1*R*,2*R*)-**1**		1.33	positive/negative
meso-**1**		4.19	
cyclohexane diamines	(B)		
trans-(1*S*,2*S*)-**2**		1.66	negative/positive
(1*R*,2*R*)-**2**		2.30	positive/negative
meso-**2**		11.99	
cyclopentane diols	(A)/(C)		
trans-(1*S*,2*S*)-**3**		1.21/15.52	negative/positive
(1*R*,2*R*)-**3**		1.21/15.03	positive/negative
meso-**3**		8.15	

[a] HPLC conditions: flow rate = 0.6 mL/min; (A) Develosil 60-3 (50 cm × 4.6 mm i.d.), eluents *n*-hexane/*t*-BuOH = 250/1; (B) Develosil 60-3 (25 cm × 4.6 mm i.d.), eluents *n*-hexane/*t*-BuOH = 20/1. (C) Develosil 60-3 (50 cm × 4.6 mm i.d.), eluents *n*-hexane/*t*-BuOH = 750/1. [b] Capacity factor.

As in the typical models, a series of stereoisomeric 1,2-diols and 1,2-diamines **1**−**3** were employed (Table 1). They were derivatized with (*S*)-TBMB carboxylic acid in an established manner[13] and subjected to the off-line and/or on-line HPLC−CD analyses. The off-line CD studies of di-(*S*)-TBMB carbonyl derivatives of (1*R*,2*R*)-cyclohexane diol [(1*R*,2*R*)-**1**] and diamine [(1*R*,2*R*)-**2**] indicated that strong exciton CD curves were induced by the derivatization (Figure 1). The bisignate CD peaks matched with the "dibenzoate chirality rule"[2]; the anticlockwise helicity of the vicinal -OH and -NH_2 groups gave a negative peak at *ca*. 225 nm and a positive one at *ca*. 215 nm. The above CD data as well as those of sugar derivatives in our previous report[14] support the conclusion that the (*S*)-TBMB carbonyl chromophore [$\pi-\pi^*$ transition at *ca*. 220 nm] can be used for the dibenzoate chirality method in a similar manner to symmetrical *p*-substituted benzoyl chromophores. Moreover, the much smaller CD of the agent itself [$[\theta]_{220} < +3000$] compared with the strong exciton CD will not disturb the CD analysis seriously.

HPLC analyses of a mixture of 1,2-*trans* and *cis*-isomers of **1**, derivatized with (*S*)-TBMB carboxylic acid, showed that the three possible stereoisomers could be well separated on a normal phase silica gel column (Figure 2 and Table 1). By using the fluorescence detector [excitation at 315 nm and emission at 380 nm], these stereoisomers could be detected in amounts less than 0.05 p mol per injection onto the column. When each of the HPLC eluents was monitored by the on-line CD fixing the wavelength at either 215 or 225 nm, each showed unique CD response reflecting their absolute structures (Figure 2). The (1*S*,2*S*)-isomer and its antipodal (1*R*,2*R*)-isomer showed nega-

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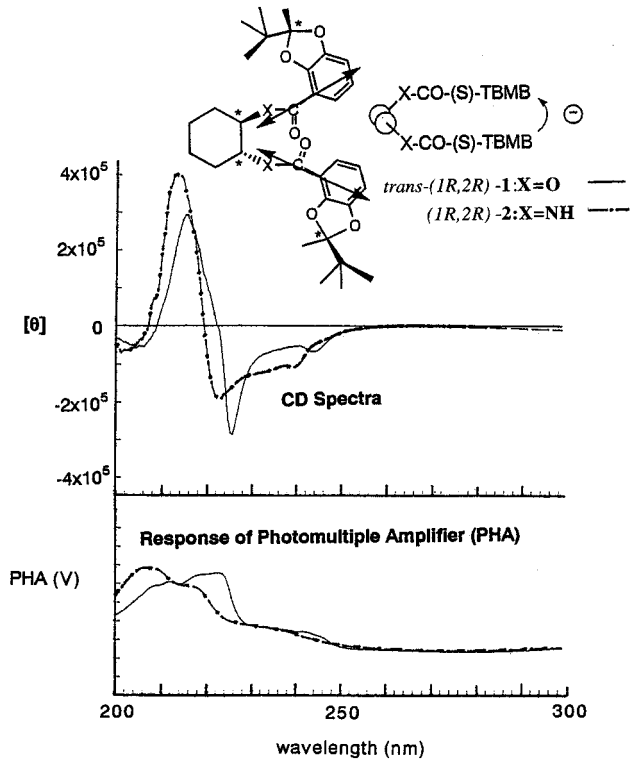

Figure 1. CD spectra of di-(*S*)-TBMB carbonyl derivatives of (1*R*,2*R*)-cyclohexane diol **1** and (1*R*,2*R*)-diamine **2** in ethanol.

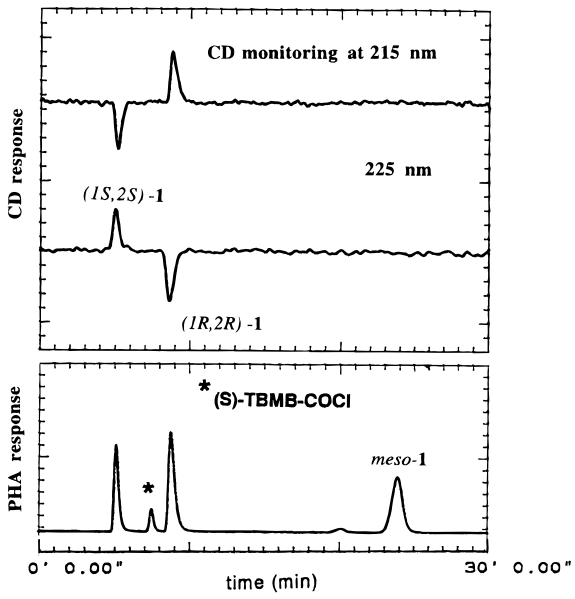

Figure 2. On-line HPLC−exciton CD analysis of 1,2-cyclohexane diols **1** (HPLC condition is cited under Table 1).

tive and positive CD response, respectively, at 215 nm, and the signs were reversed by switching the wavelength between 215 and 225 nm. The CD response of the symmetrical 1,2-*cis*(*meso*)-isomer as well as that of the reagent [(*S*)-TBMB carbonyl chloride] was negligibly small, and their HPLC peaks, observable by the fluorescence detector, completely disappeared on the HPLC-CD chromatogram. The on-line HPLC−CD analyses of **2** showed similar results except that a much better separation among the three stereoisomers was obtained (Table 1).

Another notable result was obtained in the analysis of 1,2-cyclopentane diol **3**. In this case, HPLC separation of the two

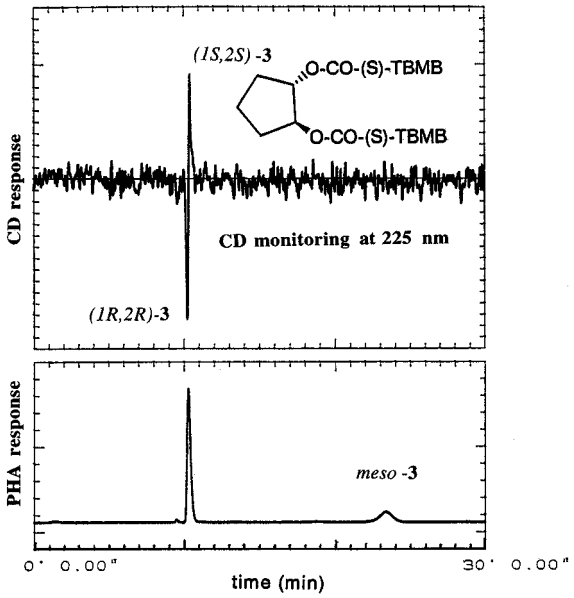

Figure 3. On-line HPLC−exciton CD analysis of 1,2-cyclopentane diols **3**.

1,2-*trans*-isomers could not be simply achieved. Figure 3 shows, however, that the overlapping peaks of the 1,2-*trans*-isomers are split into two peaks with opposite signs. This means that the CD detection can catch a slight difference in the elution order. When the polarity of the HPLC solvents is decreased, the two isomers can be better separated (separation factor $\alpha = 1.03$, Table 1), keeping the same elution order as that shown in Figure 3. Here, it is also worth mentioning that (1*S*,2*S*)-**3** elutes after (1*R*,2*R*)-**3**, while (1*S*,2*S*)-**1** elutes before (1*R*,2*R*)-**1** under nearly the same HPLC condition, although compounds **1** and **3** have structures analogous to each other. This unexpected result shows that the elution order of an enantiomeric pair may be reversed from that of the authentic compounds due to the slight difference in the structure and/or in the HPLC condition. Thus, the use of elution orders to determine the absolute configuration should be approached very cautiously and confirmed by an independent method such as CD, as is carried out in the present method.

In conclusion, we have demonstrated an on-line HPLC−exciton CD method employing the versatile chiral benzoyl agent, (*S*)-TBMB carboxylic acid. This new approach allows us to determine both the absolute configurations and the enantiomeric compositions of asymmetric 1,2-diols and 1,2-diamines in a simultaneous manner. Thus, this method represents a powerful tool for screening natural or synthetic compounds bearing these functional groups. Moreover, similar approaches will be possible also for the other types of asymmetric alcohols and amines such as acyclic polyols,[3−10] allylic alcohols,[15,16] and the other unsaturated alcohols for which exciton CD chirality methods[2] are applicable. The extension of the present method is underway.

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