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Use of a racemic derivatizing agent for measurement of enantiomeric excess by circular dichroism spectroscopy

Tetsutaro Hattori,[a,*] Yuji Minato,[a] Sulan Yao,[b] M. G. Finn[b,*] and Sotaro Miyano[a]

[a]*Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Aramaki-Aoba 07, Aoba-ku, Sendai 980-8579, Japan*

[b]*Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA*

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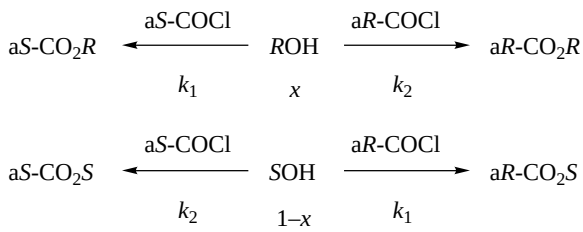
Abstract—Enantiomeric excesses of several alcohols and an amine have been determined by derivatization with *racemic* 2′-methoxy-1,1′-binaphthyl-2-carbonyl chloride *rac*-**1**, *with no requirement for kinetic resolution* in the acylation step. The necessary information is provided instead by CD and UV spectra of the derived esters and amides, the chiral signal being dominated by the binaphthyl component.

Dramatic progress in preparation methods for optically active compounds calls for efficient ways to determine their optical purities. Although chiral HPLC and GC analyses have become the standard methods for determining enantiomeric purity, the physical separation of a pair of enantiomers is generally time-consuming and cannot be accomplished in some cases. Therefore, alternative techniques which do not require enantiomer separation are useful. Circular dichroism (CD) spectroscopy, as well as polarimetry, can measure the concentration difference between the enantiomer pair in a mixture and has been used to determine the enantiomeric excess in conjunction with UV spectroscopy, which can measure the total concentration of the two enantiomers.[1,2] A general drawback of this method is its poor applicability to compounds having low CD intensities. This has been addressed in several reported cases by derivatization with achiral chromophoric reagents.[3,4] Induced CD exhibited by the ligand exchange reaction of a metal complex and by the formation of an inclusion compound have also been used for this purpose,[5] but the nonlinear relationship between the CD intensity and the enantiomeric purity often makes the method complicated.[6] Herein, we describe a new method employing a racemic chromophoric reagent for the determination of enantiomeric excesses of alcohols and amines by the combination of CD and UV spectroscopies.[7] Although optically pure derivatizing agents have been widely used in a variety of spectroscopic or chromatographic methods,[8] this may be the first example of the use of a racemic derivatizing agent.

One of us has recently reported a method to determine enantiomeric excesses of alcohols and amines by their mass spectroscopic analyses after derivatization with a large excess of a pair of *pseudo*-enantiomeric acylating agents, in which enantiomeric information is coded by mass.[9] The method requires a certain amount of kinetic resolution in the acylation, and the enantiomeric composition of the starting substrate is calculated from the relative amount of the derivatives of different molecular weights, which can be measured by mass spectroscopy. We noticed that CD spectroscopy offers the opportunity to perform a similar experiment using a truly racemic acylating agent, since CD allows the decoding of enantiomeric information without a tag of any sort.



Scheme 1. Generalized reaction of a partially optically active alcohol with a racemic acyl chloride. Legends: x, molar ratio of ROH ($0 \leq x \leq 1$); k_1, k_2, the rate constants of each reaction.

* Corresponding authors. Tel.: +81-22-217-7263; fax: +81-22-217-7293 (T.H.); tel.: +1-858-784-8845; fax.: +1-858-784-8850 (M.G.F.); e-mail: hattori@orgsynth.che.tohoku.ac.jp; mgfinn@scripps.edu

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Furthermore, as discussed below, the method is applicable *even if kinetic resolution does not occur in the derivatization process.*

Thus, an optically active alcohol, the enantiomeric components of which are designated *R*OH and *S*OH, is esterified with a large excess of a racemic acyl chloride, abbreviated as a*S*-COCl and a*R*-COCl, to give two sets of diastereomeric esters (Scheme 1). Assuming that these reactions are first order in the acyl chloride and the alcohol, the molar ratio of the esters is given by Eq. (1), where *s* is the kinetic resolution selectivity factor. Assuming no intermolecular interactions which perturb their CD behavior, the molar CD observed for the ester mixture is given by the weighted average of the molar CDs of its components, and a pair of the enantiomers show the same CD with opposite signs. Therefore, the molar CD of the ester mixture is the product of the enantiomeric excess of the starting alcohol and the molar CD of the ester mixture derived from (*R*)- [or (*S*)-] alcohol of 100% ee, as shown in Eq. (2).

$$[a\mathit{R}\text{-}CO_2\mathit{R}]:[a\mathit{S}\text{-}CO_2\mathit{R}]:[a\mathit{S}\text{-}CO_2\mathit{S}]:[a\mathit{R}\text{-}CO_2\mathit{S}] = \frac{x}{1+s}:\frac{sx}{1+s}:\frac{1-x}{1+s}:\frac{s(1-x)}{1+s} \quad \left(s=\frac{k_1}{k_2}\right) \tag{1}$$

$$\Delta\varepsilon_{obs} = (2x-1)\left\{\frac{s}{1+s}\,\Delta\varepsilon(a\mathit{S}\text{-}CO_2\mathit{R}) + \frac{1}{1+s}\,\Delta\varepsilon(a\mathit{R}\text{-}CO_2\mathit{R})\right\} = \frac{\%\ ee}{100}\,\Delta\varepsilon_{100} \tag{2}$$

The method is most generally applicable with acyl derivatizing agents having characteristically strong CD signals, such that the CD of their derived esters are dominated by the acyl component. Two situations are then possible. (a) If the CD spectra of the esters are due *only* to the acyl component, then the CD spectra of a*S*-CO_2R and a*S*-CO_2S are the same (and exactly opposite to the spectra of a*R*-CO_2R and a*R*-CO_2S). Some level of kinetic resolution is therefore necessary ($s \neq 1.0$), and the situation is directly analogous to the mass spectrometric method. (b) If the CD spectra of the esters are dominated by the acyl component but also influenced to some extent by the alcohol component, then the CD spectra of a*S*-CO_2R and a*S*-CO_2S, while of the same direction, will not be identical. It should then be possible to deduce the enantiomeric ratio of starting alcohols from the CD spectra of the derived esters for any *s* value, including situations when no kinetic resolution is obtained in the acylation step ($s = 1.0$). As with the MS methodology, a calibration measurement is necessary using a sample of known enantiomeric excess, preferably a pure enantiomer. In contrast to the MS technique, the concentration of the product esters must be taken into account, conveniently done with the UV–vis absorption spectrum obtained during the CD measurement.

We have employed 2′-methoxy-1,1′-binaphthyl-2-carbonyl chloride, *rac*-**1**, as the derivatizing agent because of the intense Cotton effects originating from exciton interaction between the two naphthalene chromophores. In addition, the acid can be readily prepared in both racemic and optically pure forms by using an ester-mediated S_NAr protocol.[10,11] Fig. 1 shows the CD and UV spectra of the optically pure (a*S*,*R*)- and (a*S*,*S*)-1-phenylethyl esters, revealing this to be an example of case (b) above. Both spectra show the positive first and the negative second Cotton effects corresponding to the clockwise twist of the binaphthyl axis, but the spectra are not completely identical due to participation of the chirality of the 1-phenylethyl moiety in the exciton interaction. On the other hand, their UV spectra are identical within experimental errors. The *s* value can be experimentally derived from the ratio of peaks obtained in HPLC analysis of the esters derived from the racemic acyl chloride on an achiral column, showing [a*S*-CO_2R]+[a*R*-CO_2S] versus [a*S*-CO_2S]+[a*R*-CO_2R].

Thus, 1-phenylethanol of known enantiomeric excess listed in Fig. 2 was esterified with 20 equiv. of the acyl chloride *rac*-**1** and the product ester mixture was analyzed by HPLC after purification by column chromatography.[12] The *s* value of the acylation reaction was found to be 1.03–1.06.[14] Fig. 2 compares the measured CD spectra with the calculated ones assuming that $s = 1.0$. The close correspondence of the curves validates the assumptions of the method and shows that the technique is not very sensitive to the magnitude of *s* for cases in which kinetic resolution selectivity is poor. Such cases will be in the majority, since effective kinetic resolution of this kind is difficult to achieve.

As the molar absorptivities of the diastereomeric esters are identical as shown in Fig. 1, a dual detector to

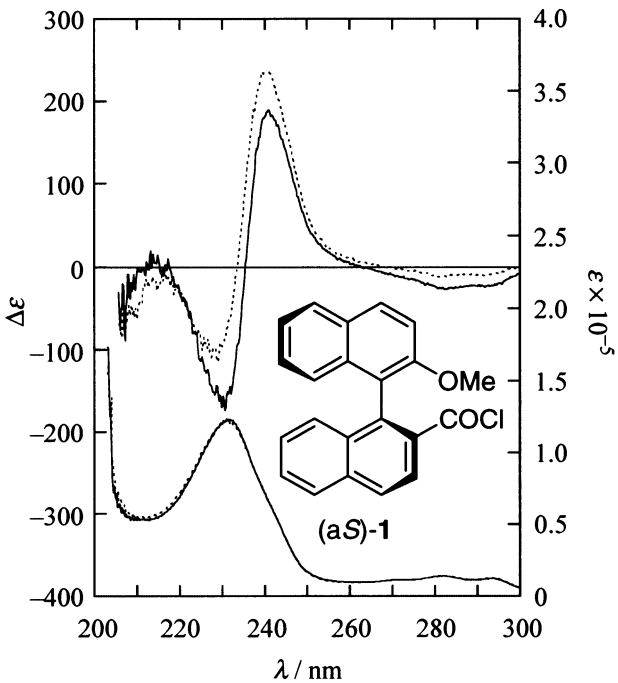

Figure 1. CD and UV spectra of (*R*)-1-phenylethyl ester of (a*S*)-**1** (solid curves) and those of (*S*)-1-phenylethyl ester of (a*S*)-**1** (dotted curves). Solvent: ethanol–1,4-dioxane (9:1).

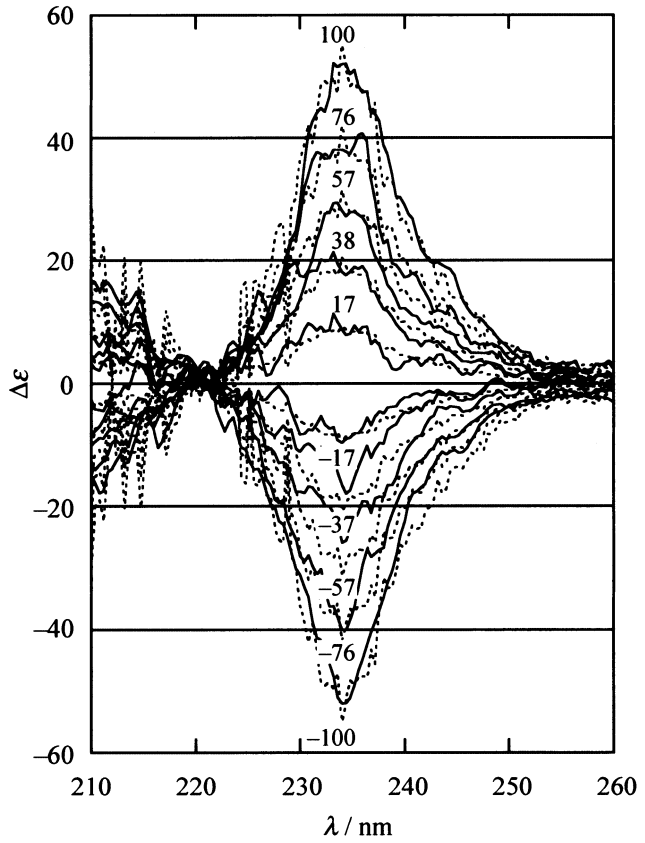

Figure 2. Measured (solid curves) and calculated (dotted curves) CD spectra of the ester mixtures derived from *rac*-**1** and 1-phenylethanol samples of the indicated optical purities. The minus sign denotes a sample enriched in the (*S*)-alcohol. Solvent: ethanol–1,4-dioxane (9:1).

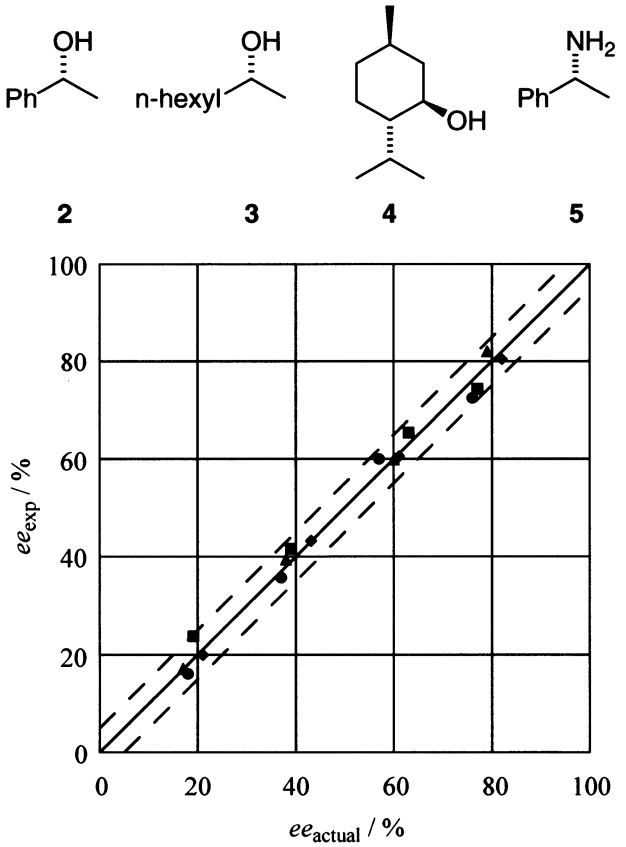

Figure 3. Plots of the actual versus measured enantiomeric excesses. Legend: **2** (●); **3** (◆); **4** (▲); **5** (■).

gather both CD and standard absorption data is advantageous.[1] With such instrumentation, a practical procedure to determine enantiomeric excess is as follows.[12] First, the CD and UV spectra are measured for the calibration sample derived from an alcohol of 100% ee and the maximum value of the elliptic angle is divided by the maximum value of the absorbance. The same operations are then repeated for the analyte of unknown % ee and concentration. The enantiomeric excess of the analyte is calculated by substituting the two quotients in Eq. (3). According to this procedure, compounds **2**–**5** of different enantiomeric compositions were analyzed (Fig. 3). In every case, the measured value fell within 5% ee of the actual value.[15] Interestingly, the procedure failed for the analysis of enantiomerically enriched 2-octylamine, because its diastereomeric amides showed exactly the same CD spectra. This is therefore an example of case (a) above, with a kinetic resolution selectivity ($s=0.94$) insufficient to allow a determination of ee to be made.[16]

$$\frac{\theta_{obs}}{A_{obs}}=\frac{\%\ ee}{100}\cdot\frac{\theta_{100}}{A_{100}} \qquad (3)$$

Circular dichroism spectroscopy therefore frees us from the need to code enantiomeric information into the derivatizing agent[9] or the substrate,[17] and from the need to accomplish derivatization in an enantioselective manner. Instead, the necessary information is provided by a combination of the dominating CD signal of each enantiomer of the racemic 'reporter' structure (binaphthyl acyl in this case) and a perturbation of that signal provided by each enantiomer of the analyte (alcohol or amine). By derivatizing with a potent chiral chromophore, the inherent optical rotation of the analyte does not matter, and the measurement is made far more sensitive than standard polarimetry, thereby extending the optical determination of enantiomeric purity to samples that do not exhibit strong optical rotation. A practical disadvantage of the method relative to the related MS technique is that CD measurements must be performed on purified samples so that the relative absorption values are accurate. Further studies to improve the scope and sensitivity of the method are in progress.

Acknowledgements

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12. Typical procedure for the determination of enantiomeric excess: To a solution of acyl chloride *rac*-**1** (69.4 mg, 200 μmol) in dry benzene (10 mL) was added a solution of 1-phenylethanol (20 mM in benzene; 500 μL, 10 μmol), pyridine (100 μL) and *N*,*N*-dimethyl-4-aminopyridine (12.1 mg, 99.0 μmol) and the mixture was stirred at room temperature for 24 h. After dilution with benzene, the mixture was washed successively with 2 M HCl, 1 M Na_2CO_3 and water, dried over $MgSO_4$ and evaporated. The residue was purified by TLC (silica gel) with hexane–ethyl acetate (5:1) as the developer to give the corresponding ester (3.3 mg, 76%),[13] which was dissolved in ethanol–1,4-dioxane (9:1) and analyzed by CD and UV spectroscopies using a 1 mm cell. The concentration of the sample was roughly adjusted so that the maximum absorbance ranged between 0.7 and 1.6. The CD spectrum was measured on a JASCO J-720 spectropolarimeter in the wavelength range of several nanometers where the elliptic angle became maximum. The measurement conditions were as follows: band width 0.5 nm, resolution 0.2 nm, response 2 s, scan 100 times.
13. As Eq. (1) applies to any point in the derivatization reaction, the yield does not affect the analytical accuracy.
14. The HPLC analysis was carried out on a system equipped with a silica gel column and a UV detector set at 254 nm, the eluent being 1% 2-propanol in hexane. The observed peak ratio was corrected for the molar response, which was reported to be (a*S*,*R*)-ester:(a*S*,*S*)-ester = 1.03:1 (see Ref. 11a).
15. The *s* values of the other analytes were also determined: menthol, 1.1; 2-octanol, 1.1; 1-phenylethylamine, 0.94. The values are uncorrected for the molar response.
16. We do not yet know the level of kinetic resolution selectivity needed to provide accurate ee measurements for case (a) analytes; for MS analysis, *s* should be greater than 1.2 or less than 0.83 (see Ref. 9a).
17. For example, see: Reetz, M. T.; Becker, M. H.; Klein, H.-W.; Stöckigt, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1758.