Rapid enantiomeric determination of α-hydroxy acids by electrospray ionization tandem mass spectrometry

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Direct chirality measurement of tartaric and other α-hydroxy acids at very low enantiomeric excess (ee) using a fast new mass spectrometric method.

Tartaric acid is a special molecule in the history of chirality. As the first compound resolved[1] it triggered the concept of molecular chirality and for more than 150 years it has been used to explore new chiral technologies. For instance, tartaric acid has recently been absorbed on a copper surface to study heterogeneous enantioselectivity.[2] Herein, tartaric acid, along with other α-hydroxy acids, is employed as a model compound to study chiral analysis by mass spectrometry. Previously, ester derivatives of tartaric acid, although not the acid itself, were extensively studied for gas-phase chiral recognition.[3–5] These studies, along with most other mass spectrometric experiments attempting to achieve gas-phase chiral recognition,[6,7] were qualitative. We report the first enantiomeric quantification of tartaric acid and other α-hydroxy acids in the gas phase, on the basis of a newly developed method[8,9] that employs cluster ions comprised of the analyte, a chiral reference and a transition metal. Chiral discrimination is achieved in the dissociation of these cluster ions and evaluated by the kinetic method[10,11] that is sensitive to small energy differences between diastereomers. Two independent parallel reactions are used to monitor the chiral distinction and measurements of the ratio of product ion abundances allow the quantification of enantiomeric mixtures, even at low enantiomeric excess (ee). This simple chiral analysis method employs a standard commercial instrument.[12]

Electrospray ionization (ESI) was performed on an aqueous methanol solution containing a mixture of an analyte (α-hydroxy acid, A, as an enantiomeric mixture A_R and A_S, 100 μM), a chiral reference compound (chiral amino acid, ref*,100 μM), and a transition metal ion (Co(II), 25 μM). The electrosprayed solution formed abundant singly-charged cluster ions $[Co^{II}(ref^*)_2(A) - H]^+$ which were mass-selected and dissociated in a quadrupole ion trap to competitively form the dimeric complexes $[Co^{II}(A)(ref^*) - H]^+$ and $[Co^{II}(ref^*)_2 - H]^+$ by the loss of neutral reference compound, ref*, and analyte, A, respectively. The difference in stability of the diastereomeric ions $[Co^{II}(A)(ref^*) - H]^+$ due to the two configurations of the analyte A, results in different abundances, relative to the abundance of the $[Co^{II}(ref^*)_2 - H]^+$ ion. The relative abundance ratio R (eqn. 1) depends on the enantiomeric composition of the analyte, A:

$$R = [Co^{II}(A)(ref^*) - H]^+ / [Co^{II}(ref^*)_2 - H]^+ \quad (1)$$

When the analyte is enantiomerically pure, R equals R_R or R_S. Therefore, the ratio of R_R to R_S, defined as R_{chiral}, measures the degree of chiral distinction:[13]

$$R_{chiral} = R_R / R_S = \frac{[Co^{II}(A_R)(ref^*) - H]^+ / [Co^{II}(ref^*)_2 - H]^+}{[Co^{II}(A_S)(ref^*) - H]^+ / [Co^{II}(ref^*)_2 - H]^+} \quad (2)$$

Tartaric and four other α-hydroxy acids (malic acid, mandelic acid, 3-phenyllactic acid, citramalic acid) were selected for chiral analysis. Chiral references were chosen for their capability to produce large steric interactions and for structural similarity to the analyte. Such similarity allows the complexes to form easily and it also allows accurate relative abundance ratios to be measured, otherwise dissociation proceeds overwhelmingly to form the more stable product. Amino acids (α-aminocarboxylic acids) having similar structures to the analyte (α-hydroxy acid) and the nineteen natural chiral α-amino acids, plus numerous other amino acids, provide an array of choices. Aromatic amino acids were observed to provide the greatest chiral distinction.[8,9] The use of Co(II) ion as the central ion rather than Cu(II) or Ni(II) previously used for chiral recognition of amino acids[8,9] is because of its ready binding to hydroxy groups. By contrast, Cu(II) and Ni(II) bind to amino groups so strongly that loss of the hydroxy acid is the only observed dissociation channel.

Typical spectra showing the distinction of D- and L-malic acid, using L-tyrosine as the chiral reference, are shown in Fig. 1. The chiral recognition of five α-hydroxy acids is summarized in Table 1. Abundance ratios showed standard deviations of 2%. Chiral selectivity (R_{chiral})[13] values for the five α-hydroxy acids ranged from 0.67 to 1.43. Among them, tartaric acid shows moderate chiral selectivity (R_{chiral} = 1.29). Mandelic acid shows a low affinity for Co(II) and there is no chiral distinction between *R* and *S*-mandelic acids with L-alanine as reference. However, when a chiral aromatic compound, L-3-phenyllactic acid, is used as reference, chiral distinction is observed with an

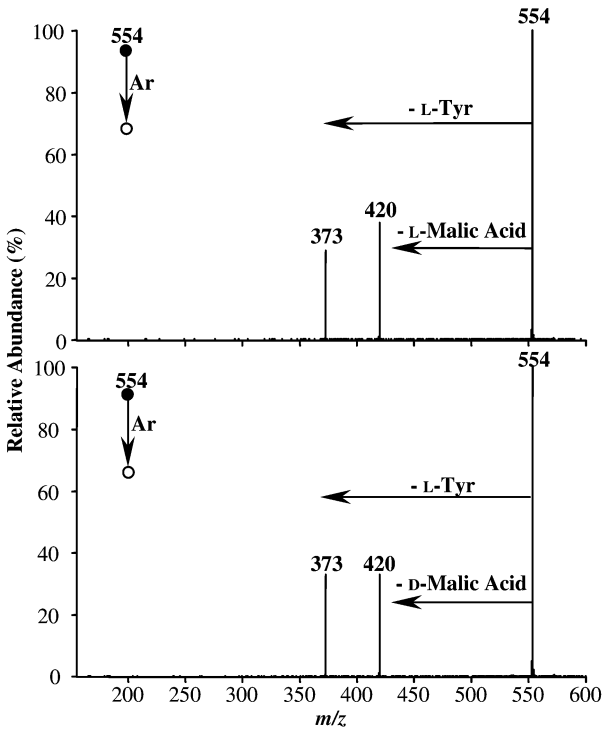

Fig. 1 MS/MS product ion spectra of (a) $[Co^{II}$(L-malic acid)$(L\text{-Tyr})_2 - H]^+$ (*m/z* 554); (b) $[Co^{II}$(D-malic acid)$(L\text{-Trp})_2 - H]^+$ (*m/z* 554). The CID activation level is chosen as 11%, corresponding to approximately 275 mV AC.

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Table 1 Chiral recognition of α-hydroxy acids.[a,b]

A	Ref*	$[Co^{II}(ref^*)(A) - H]^{+\cdot}/[Co^{II}(ref^*)_2 - H]^{+\cdot}$	R_{chiral}
D-Tartaric acid	L-Tyr	0.823	1.29
L-Tartaric acid		0.640	
D-Malic acid	L-Tyr	1.11	1.43
L-Malic acid		0.775	
R-Mandelic acid	L-Ala	1.00	1.00
S-Mandelic acid		1.02	
R-Mandelic acid	L-3-Phenyllactic acid	0.175	0.81
S-Mandelic acid		0.216	
D-3-Phenyllactic acid	L-Pro	0.168	1.29
L-3-Phenyllactic acid		0.130	
D-3-Phenyllactic acid	L-Phe-d_5	0.0226	0.67
L-3-Phenyllactic acid		0.0337	
R-Citramalic acid	L-Tyr	5.81	1.35
S-Citramalic acid		4.30	

[a] R_{chiral} is defined in eqn. 2. [b] CID activation level is optimized in each experiment and then kept constant for the measurement of enantiomers.

R_{chiral} value of 0.81. Chiral recognition of D- and L-3-phenyllactic acids shows the largest chiral effect using L-phenylalanine-d_5 as the reference (an isotopically labelled compound was used for convenience, since there is only one-dalton mass difference between phenylalanine and 3-phenyllactic acid). As expected,[8,9] when a non-aromatic compound, for example L-proline, is used as reference the chiral selectivity is smaller.

Quantification of tartaric acid was performed using an enantiomerically pure chiral reference (L-tyrosine) and tartaric acid in various ee. The experiments focused on mixtures with extreme ee values, since these are particularly difficult to analyze accurately and yet combinatorial syntheses frequently yield such samples. The ratio (*R*) of the two fragment ions was measured in a single tandem (MS/MS) spectrum as a function of the ee of the tartaric acid. A linear relationship of ln (*R*) *versus* ee was obtained (Fig. 2) with a correlation coefficient (r^2) of 0.9945. Such a linear correlation between the logarithm of the fragment ion abundance ratio and the ee is intrinsic to the kinetic method[10,11] and is the result of the logarithmic relationship between relative ion abundance and energy that characterizes this method.[14] On the basis of such an experimentally established semi-log plot, two-point calibrations can be performed using a racemic sample and a sample of known ee and quantitative chiral analysis carried out by measuring the ratio of two fragment ions in a single spectrum, within a time of about 1 min.

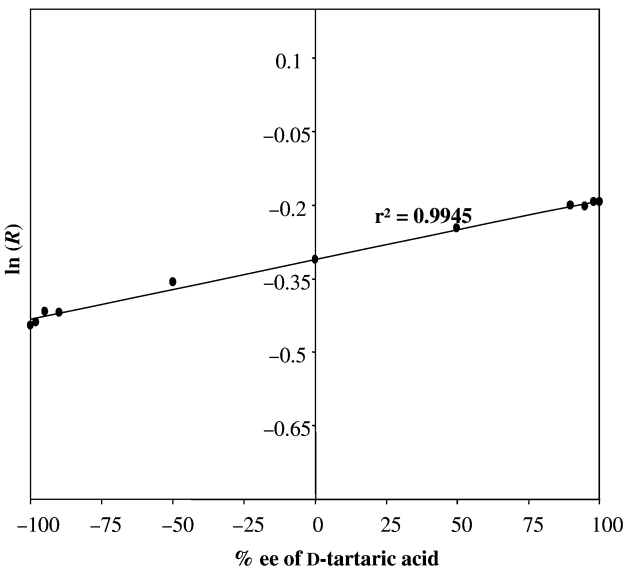

Fig. 2 Calibration curve for chiral analysis of tartaric acid using L-tyrosine as the reference.

The present study has described a novel method for rapid enantiomeric determination. At low %ee values, where the error is greatest, tartaric acid samples whose ee values differ by 2% (3 and 5%) can be distinguished at the σ confidence level in the current experiments.[15] When applying the method to real mixtures, matrix effects may influence its accuracy. Note that the chiral resolution (R_{chiral}) achieved for tartaric acid is only 1.29, and further improvement in chiral selectivity will further improve the method. The experiment, along with the previous observation of chiral analysis of other types of chiral compounds, represents a general mass spectrometric method for gas-phase chiral analysis. The measurements are simple, rapid, and only require very small amounts of sample for analysis. Further extension of this work to the study of other chiral compounds, such as chiral drugs is in progress.

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12 All experiments were performed using a commercial LCQ ion trap mass spectrometer (Finnigan, San Jose, CA), equipped with an ESI source and operated in the positive ion mode. Spectra shown represent the average of about 50 scans, each requiring 0.2 s. The sample was infused *via* a syringe pump at a flow rate of 1–2 μL min^{-1}. The collision-induced dissociation (CID) conditions were optimized for each analyte.
13 R_{chiral} is equivalently defined as the ratio of R_D to R_L (nomenclature commonly used for chiral acids). The further the R_{chiral} value is from unity, the higher the degree of chiral recognition. $R_{chiral} = 1$ indicates no chiral discrimination, which means that the particular combination of Co(II) ion and reference ligand fails to create stereochemically-distinctive interactions with the enantiomers under the observation conditions employed.
14 From the kinetic method, ln (*R*) is linearly proportional to the energy change $\Delta Co^{II}BDE\{(A)(ref^*)\}$, where $\Delta Co^{II}BDE\{(A)(ref^*)\}$ is defined as energy of the reaction $[Co^{II}(ref^*)_2 - H]^+ + A \rightarrow [Co^{II}(ref^*)(A) - H]^+ + ref^*$, and the designation $\Delta Co^{II}BDE\{(A)(ref^*)\}$ is used to recognize that the energy term involves both deprotonation and binding to Co. The quantity $\Delta Co^{II}BDE\{(A)(ref^*)\}$ is linearly proportional to the ee of the analyte A, therefore ln (*R*) changes linearly with ee. Detailed derivation is available in ref. 9.
15 Multiple injections were used to determine these values which refer to standard deviation σ.