Resolution by Sublimation

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Sublime Arguments: Rethinking the Generation of Homochirality under Prebiotic Conditions**

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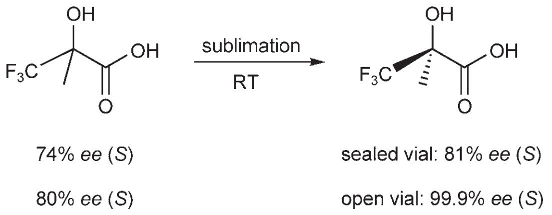
Chirogenesis, the study of the origin and evolution of the homochiral homogeneity found in living organisms on earth, has become a fascinating and controversial conundrum of modern science. Scientific literature and cutting-edge research seem to be saturated with the terms chirality and asymmetry. This not only reflects the intimate link between the search for the origin of homochirality and the origin of life itself, but also the inherent importance of related areas such as enantioselective synthesis and asymmetric catalysis in both academia and industry.

While we should certainly reconsider the chirogenesis of small molecules and polymers in terms of substrates and processes that would be plausible in a prebiotic scenario,[1] no one has come up with an explanantion more suitable than stochastic spontaneous resolution.[2] Amplification of the very small imbalance in a mixture of handed molecules is guided by basic principles in stereochemistry and physical chemistry, such as the nature and properties of the racemic state, the eutectic compositions for enantiomers, and other thermodynamic and kinetic considerations, which have often been overlooked, if not ignored, by the scientific community. It is not trivial to emphasize that crystalline forms of chiral substances occur as conglomerates, racemic compounds, and even solid solutions.[3,4] Trace imbalances of one enantiomer within a mixture of enantiomers do effectively create diastereomeric relationships that have profound implications and can be harnessed further in resolution protocols. Overall, evolutionary homochirality appears to be a stereochemical imperative.[5]

These premises are a suitable context for discussing a series of reinvestigations of stereoselective sublimations that led to essentially enantiomerically pure compounds starting from an enantiomerically enriched material.[6,7] Thus, it was observed that a sample of (*S*)-α-(trifluoromethyl)lactic acid, which initially had an enantiomeric excess of 74 %, increased to 81 % *ee* when it stored in a sealed vial and partial sublimation had occurred. Notably, the sublimed material was only of 35 % *ee* (Scheme 1).

To further corroborate fractional sublimation as the source of enantioenrichment, Soloshonok and associates conducted other experiments in open air with enantioenriched samples of the lactic acid derivative. They obtained reproducible results in which the enantiomeric purity of the sublimate was invariably lower than that of the original sample, while the optical purity of the remainder substantially increased. Finally, starting from a sample with 80 % *ee* in an open vial at room temperature, they were able to obtain the enantiomerically pure acid after 56.5 h.



Scheme 1. Sublimation of α-trifluoromethyllactic acid with concomitant improvements of enantiomeric excess.

As pointed out before, the big idea behind all this work is that the vapor pressure of enantiomers and of their mixtures may be markedly different and may chiefly depend on the enthalpy of decomposition of the racemic compound and on the temperature at which this process occurs.[8,9] In other words, striking differences in intermolecular interactions in enantiomers and racemates account for mechanisms capable of producing spontaneous enantioseparations, even with "achiral" physical influences. In addition, such differences constitute a manifestation of *stereoisomer discrimination*, which should be, as recognized by leading stereochemists, one of the major challenges of modern stereochemistry.[10] Data on heats of sublimation reported in the early 1980s indicated that heterochiral interactions between the enantiomers can be either stronger or weaker than the corresponding homochiral interactions.[11]

To shed light upon the "self-purification" of α-trifluoromethyllactic acid,[6] Soloshonok and co-workers further investigated the molecular arrangement and packing in

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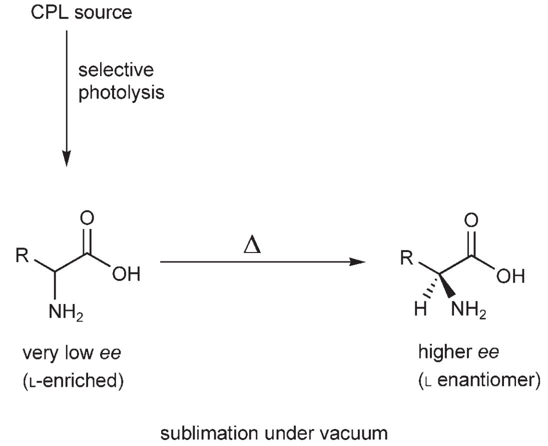
crystals of the racemate and of one enantiomer. While the crystal lattice of the racemate is composed of heterochiral dimers with two hydrogen bonds between *R* and *S* enantiomers, the molecules in the crystal of the *S* enantiomer are arranged to form four hydrogen bonds with other four adjacent molecules. Moreover, the F···F contact of the CF_3 groups in the racemate is closer than that in the enantiomerically pure crystal, for which no appreciable interactions are observed.

These sublimation protocols are certainly not new. More than four decades ago, pioneering work revealed that fractional sublimation of some enantioenriched substances, including phenylalanine derivatives, provides fractions of increased enantiomeric purity.[9,12] However, these and other researchers did not completely understand the variables and mechanisms involved in this resolution. It is the composition of the eutectic, with respect to that of the initial mixture, that dictates the preferential sublimation.[9] It is important to note that, contrary to a long-held belief, the modification with the lowest melting point is not necessarily the one that sublimes first.[13] It is equally noteworthy that sublimation not only of partially enriched samples of racemic compounds but also of conglomerates and pseudoracemates can result in self-purification.[14] These aspects suggest that the enhancement of optical activity attained by such thermal processes could be more efficient than, or at least an alternative to, spontaneous crystallization of suitable compounds.[12c]

But if sublimation did effectively play a role in the primeval separation of chiral building blocks, then amino acids should also be considered. Two recent studies resembling that of Soloshonok et al. deal with proteinogenic amino acids. Thus, Cooks and co-workers reported that sublimation of near-racemic samples of serine (3% *ee*, L-enriched) yielded a sublimate that was highly enriched in the major enantiomer (>68% *ee* obtained at temperatures between 190 and 205°C).[15] Remarkably, no selectivity was found with other amino acids such as alanine. Since serine undergoes enantioenrichment, one necessarily recalls formation of homochiral clusters in the gas phase,[16] which could then account for the chirogenesis. However, the magically charged clusters appear to hold for serine only and are generated under ionizing conditions, a fact also observed for other chiral substances by mass spectrometry.[17]

In a subsequent publication, Feringa and associates found that a series of L-enriched amino acids (leucine, phenylalanine, alanine, valine, methionine, and serine) gave sublimates of higher enantiomeric excess upon heating in a sublimation apparatus under vacuum (Scheme 2).[18] The authors claim the astrophysical relevance of this experiment, as amino acids with low enantiopurity may be generated by ubiquitous physical fields of our visible universe such as circularly polarized light (CPL). For instance, L-leucine having 1% *ee* gave rise to a sublimate with 39% *ee* after 21 h.



Scheme 2. Enantiomeric enrichment of amino acid sublimates.

Clearly these independent research groups provided different results, but can their findings be reconciled by invoking well-established basic science? This aspect has been scrutinized by Blackmond and Klussmann in an excellent account on phase-behavior models,[1a] and the readership is therefore directed to this contribution for an in-depth assessment. However, some fundamental arguments will be mentioned here that reveal again the distinctive behavior and fate of different racemic solids. One should first mention a note of caution as the Cooks and Feringa experiments were most likely carried out under far from equilibrium conditions and, in addition the sublimation temperatures were different. In any event, both groups essentially observed eutectic behavior of the amino acids, which holds also true in the previously mentioned Soloshonok experiments.[6]

In principle, an enantioenriched sublimate should arise from an amino acid that forms a racemic compound at a composition with an *ee* value below the one of its eutectic. In fact, the sublimation *ee* values agree well with the eutectic values found for these amino acids in solution (e.g. >99% for serine at equilibrium), thus showing a good parallelism between crystal solubility and volatility. What is noticeable is that the two groups prepared the enantioenriched samples in different ways. Cooks et al. mixed slightly different amounts of pure D and L amino acids, whereas Feringa et al. mixed the racemic D/L compound with an excess of the L enantiomer. In the latter case, solid–solid equilibration of, for instance alanine, had taken place prior to sublimation and the composition would thus reflect an *ee* value close to that of the eutectic point. But alanine prepared from separate D and L crystals behaves rather as a conglomerate before solid–solid equilibration of the solid D/L compound is attained. Blackmond coined the term *kinetic conglomerate* for this situation.[1a,19] It is convenient to underline that gas-solid and solution-solid phase transitions are not directly comparable, although both processes may lead to enantioenriched samples of amino acids and other relevant molecules.[1a,20,21]

It seems that sublimation should be reasonably regarded as a plausible mechanism for the formation of optically active crystals. After all, the crystal state provides an appropriate and stable means of maintaining the chiral integrity of chiral molecules.[1,2b,22] Subsequent stochastic sorting of crystals of optically enriched or enantiopure compounds, easily conducted by natural agents, might have generated highly enantiopure niches in the prebiotic world.[23]

Using little more than simple concepts and experiments, several research groups have focused on substrates of biological importance and have made a big step toward understanding many perplexing evolutionary processes that give rise to homochirality. The bottom-line message is now clear: Although our interest in the emergence of homochiral sequences should be maintained, such phenomena are consistent with predictable stereochemistry and thermodynamics. This is actually more important than a touch of magic.

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[1] For recent and insightful discussions focused on amino acids and peptides: a) D. G. Blackmond, M. Klussmann, *Chem. Commun.* **2007**, 3990 – 3996; b) "Prebiotic Chemistry": I. Weissbuch, L. Leiserowitz, M. Lahav, *Top. Curr. Chem.* **2005**, *259*, 123 – 165, and references therein; c) J. G. Nery, R. Eliash, G. Bolbach, I. Weissbuch, M. Lahav, *Chirality* **2007**, *19*, 612 – 624.

[2] a) J. S. Siegel, *Nature* **2002**, *419*, 346 – 347; b) L. Pérez-García, D. B. Amabilino, *Chem. Soc. Rev.* **2007**, *36*, 941 – 967, and references therein.

[3] a) *Conglomerates* accounting for 10–15 % of known molecules, are simply a 1:1 mechanical mixture of crystals of the two enantiomers, each crystal made of homochiral molecules. The term *racemic mixture* has also been applied to conglomerates, but this expression should be avoided today. *Racemic compounds*, also called *racemates*, represent a majority and consist of crystals, each containing the two enantiomers in a 1:1 ratio down to the unit-cell level. Solid solutions of the two enantiomers, also denoted as *pseudoracemates*, are quite rare and consist of an unordered 1:1 stoichiometric mixture of both enantiomers in the solid phase. b) The reader is also referred to the IUPAC document on stereochemistry terminology: G. P. Moss, *Pure Appl. Chem.* **1996**, *68*, 2193 – 2222.

[4] The existence of conglomerates and racemic compounds has been explained in terms of *enantiophobic* and *enantiophilic* molecules, respectively, thus reflecting the distinctive nature of packing forces; see Ref. [2b] for an extended discussion.

[5] J. S. Siegel, *Chirality* **1998**, *10*, 24 – 27.

[6] V. A. Soloshonok, H. Ueki, M. Yasumoto, S. Mekala, J. S. Hirschi, D. A. Singleton, *J. Am. Chem. Soc.* **2007**, *129*, 12112 – 12113.

[7] The title of this article contains the expression *Chiral Non-Racemic Compounds*, whose use engenders ambiguity and is to be discouraged (as the sample may not be enantiomerically pure). For the sake of clarity, see: E. L. Eliel, S. H. Wilen, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**, pp. 5 and 214.

[8] M. Farina, *J. Chem. Soc. Chem. Commun.* **1987**, 1121 – 1122.

[9] For a detailed discussion: J. Jacques, A. Collet, S. H. Wilen, *Enantiomers, Racemates, and Resolutions*, Krieger, Malabar, FL, **1981**, pp. 159 – 165.

[10] An excellent overview of stereoisomer discrimination, highlighting the distinctive properties of stereoisomers, can be found in: E. L. Eliel, S. H. Wilen, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**, pp. 153 – 208.

[11] J. S. Chickos, D. L. Garin, M. Hitt, G. Schilling, *Tetrahedron* **1981**, *37*, 2255 – 2259.

[12] a) G. Pracejus, *Justus Liebigs Ann. Chem.* **1959**, *622*, 10 – 22; b) H. Kwart, D. P. Hoster, *J. Org. Chem.* **1967**, *32*, 1867 – 1870; c) D. L. Garin, D. J. C. Greco, L. Kelley, *J. Org. Chem.* **1977**, *42*, 1249 – 1251.

[13] In Ref. [6], racemic crystals sublimed faster than either enantiomer following zero-order kinetics.

[14] Conceptually, however, an ideal solid solution of two enantiomers should be unaffected by sublimation; see E. L. Eliel, S. H. Wilen, *Stereochemistry of Organic Compounds*, Wiley, New York, **1994**, p. 182.

[15] R. H. Perry, C. Wu, M. Nefliu, R. G. Cooks, *Chem. Commun.* **2007**, 1071 – 1073.

[16] S. C. Nanita, R. G. Cooks, *Angew. Chem.* **2006**, *118*, 568 – 583; *Angew. Chem. Int. Ed.* **2006**, *45*, 554 – 569.

[17] H. M. Fales, G. J. Wright, *J. Am. Chem. Soc.* **1977**, *99*, 2339 – 2340.

[18] S. P. Fletcher, R. B. C. Jagt, B. L. Feringa, *Chem. Commun.* **2007**, 2578 – 2580.

[19] M. Klussmann, S. P. Mathew, H. Iwamura, D. H. Wells Jr. , A. Armstrong, D. G. Blackmond, *Angew. Chem.* **2006**, *118*, 8157 – 8160; *Angew. Chem. Int. Ed.* **2006**, *45*, 7989 – 7992.

[20] For instance, solutions of D- or L-phenylalanine, of as little as 1 % *ee*, may be enriched up to 90 % *ee* simply when solvent is removed successively by evaporation such that the racemate precipitates selectively: R. Breslow, M. S. Levine, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 12979 – 12980.

[21] Soloshonok and co-workers have also observed the trend of some racemates to self-disproportionate in achiral chromatographic phases with achiral eluents resulting in enantioenrichment: a) V. A. Soloshonok, *Angew. Chem.* **2006**, *118*, 780 – 783; *Angew. Chem. Int. Ed.* **2006**, *45*, 766 – 769; b) V. A. Soloshonok, D. O. Berbasov, *J. Fluorine Chem.* **2006**, *127*, 597 – 603, and references therein.

[22] a) P. Cintas, *Angew. Chem.* **2002**, *114*, 1187 – 1193; *Angew. Chem. Int. Ed.* **2002**, *41*, 1139 – 1145; b) M. Avalos, R. Babiano, P. Cintas, J. L. Jiménez, J. C. Palacios, *Origins Life Evol. Biosphere* **2004**, *34*, 391 – 405.

[23] C. Welch, *Chirality* **2001**, *13*, 425 – 427.