JOC The Journal of Organic Chemistry

Note

pubs.acs.org/joc

Partial Sublimation of Enantioenriched Amino Acids at Low Temperature. Is it Coming From the Formation of a Euatmotic Composition[1] of the Gaseous Phase?

Arkadii V. Tarasevych,[†,‡] Alexander E. Sorochinsky,[‡] Valery P. Kukhar,[‡] Aurélien Chollet,[†] Richard Daniellou,[§] and Jean-Claude Guillemin*,[†]

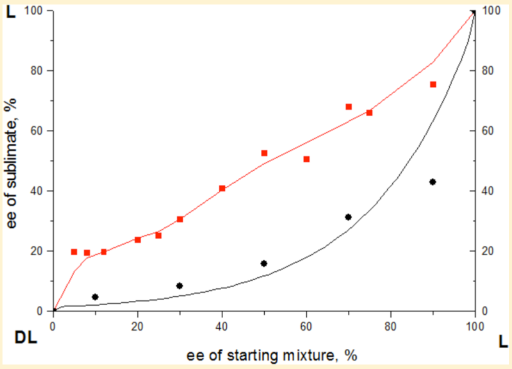
[†]École Nationale Supérieure de Chimie de Rennes, CNRS, UMR 6226, 11 Allée de Beaulieu, CS 50837, 35708 Rennes cedex 7, France

[‡]Institute of Bioorganic Chemistry and Petrochemistry, National Academy of Sciences of the Ukraine, Murmanska Street 1, 02660 Kyiv 94, Ukraine

[§]Université d'Orléans, CNRS, ICOA,UMR 7311, F-45067 Orleans 2, France

Ⓢ *Supporting Information*

ABSTRACT: The partial sublimation of enantioenriched amino acids was performed slowly at low temperature with the aim to determine the rules of sublimation of these compounds. Although the formation of a euatmotic composition of the gaseous phase starting from DL + L mixtures of Leu, Pro, and Phe can be deduced from the enantiomeric excess of sublimates, the behavior of the kinetic conglomerate explains the results for D + L mixtures of Ala, Leu, Val, and Pro. Consequently, the enantiomeric excess of the partial sublimate is dependent not only on the studied compound but also on the composition of the starting mixture.



■ INTRODUCTION

Since the enantiomeric excess (ee) of a partial sublimate and of the residue of an enantioenriched compound is almost never the same as that of the starting mixture,[2,3] the sublimation can be regarded as a potential method for enantiomeric purification.[4−6] Thus, when the ee of the sublimate is lower than the ee of the starting material, the residue in enantioenriched, and vice versa. The theoretical aspects of the solid- to gas-phase transition without any enantiomerization have been discussed at length in the literature,[7−9] and a gaseous phase with a euatmotic composition[1] has been proposed to explain the results. For compounds such as mandelic acid, lactamide, and ibuprofen[4,6,10] the equilibrium state is readily attained and a constancy of the sublimate ee over a wide range of values was revealed with experiments starting independently either from (i) nonracemic mixtures of a true racemic compound with an enantiomer or (ii) mechanical mixtures of enantiomers. The mass spectroscopic determination of relative vapor pressures of scalemic mixtures of α-(trifluoromethyl)-lactic acid revealed the same behavior: the constant enantiomeric composition of the gas phase at low and high ee values.[11]

Quite recently, reported studies on sublimation of scalemic mixtures of natural amino acids highlighted unexpected enhancements of enantiomeric excess (ee)[12,13] and reactivated the questions on the possible formation or absence of a euatmotic composition of the gas phase. *To date, except for leucine,*[14] *nobody has been able to predict the ee of a partial sublimate starting from one or a mixture of enantioenriched amino acid(s).* A great deal of disparate experimental data obtained under variable conditions that contradict in some cases the theoretically expected results pushed us to get at the root of the problem and to conduct a thorough research under conditions where careful attention is paid to the nature of the starting mixtures. In a first study devoted to the partial sublimation of leucine, we used a slow sublimation (14 h) at low temperature (100 °C) with the aim of maximum approach of equilibrium conditions.[14] We demonstrated that the increase of the ee for DL + L mixtures was not as strong as reported[12] and can be rationalized by systematic experiments.[14] In such sublimations, the formation of a euatmotic composition of the gas phase cannot be excluded but is not the only possible explanation of the experimental results. On the other hand, its formation is not observed in the slow sublimation of D + L mixtures of the same amino acid, since results correspond to the behavior of the kinetic conglomerate, the first unambiguous one reported in the literature for sublimation. Such sublimations with the

Received: August 9, 2013
Published: September 25, 2013

dx.doi.org/10.1021/jo4017474 | *J. Org. Chem.* 2013, 78, 10530−10533

condensation of the vaporized products can be quite far from the formation of a thermodynamic equilibrium and proceed in the case of mixture of enantiomers of leucine via the saturation of the gaseous phase by each enantiomer. Thus, the enantiomeric excess (ee) of the sublimate of leucine is dependent on the starting mixture. Nevertheless, studies on only one amino acid (leucine) are not enough to reach a conclusion on the behavior of sublimable amino acids and we report here a study devoted to alanine, proline, valine, and phenylalanine. We also extended this work to the sublimation of binary and ternary mixtures of amino acids with the aim of identifying some synergistic effect.

■ RESULTS AND DISCUSSION

The first studies were devoted to L + D mixtures (Figure 1) and the second studies (Figure 2) to DL + L mixtures. Many sublimations were repeated two or three times with the aim of avoiding artifacts. In each experiment, the same conditions (apparatus, pressure, time, and amount of the starting mixture) were used. Depending on the volatility of each amino acid the temperature of sublimation ranged from 75 to 140 °C, allowing slow sublimation of a small percentage of the starting mixture.

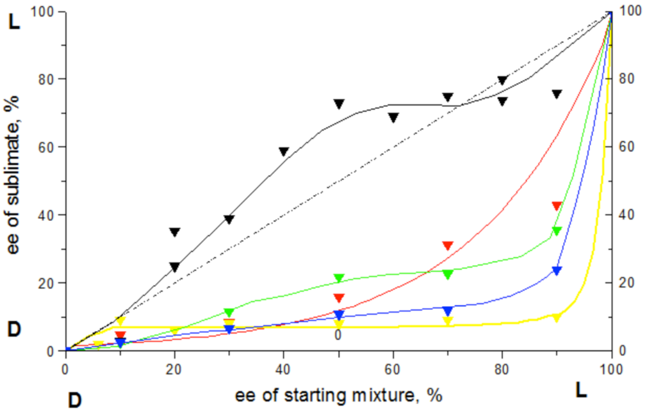


Figure 1. Enantiomeric excess of L + D mechanical mixtures of Ala (red), Leu (yellow), Phe (black), Pro (green), and Val (blue). The dashed line corresponds to the starting mixture.

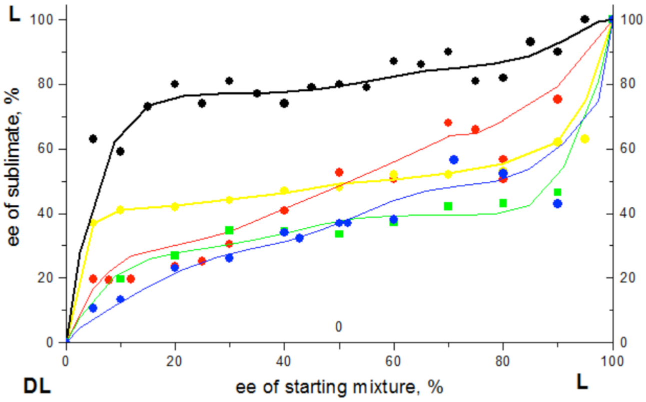


Figure 2. Enantiomeric excesses of the partial sublimate of DL + L enriched mixtures of Ala (red), Leu (yellow), Phe (black), Pro (green), and Val (blue).

The partial sublimation of mechanically mixed nonracemic samples of enantiomers of Ala, Leu, Pro, and Val (Figure 1, see also Table S1 in the Supporting Information) led to strongly enantiodepleted samples.

Most of these results are consistent with the concept of a kinetic conglomerate: the independent sublimation of each enantiomer was only controlled by its own vapor pressure.[14,15] Such a behavior leads to an enantioenriched residue. We conducted experiments for the verification of the "double solubility rule" proposed by Meyerhoffer at the beginning of the last century[17] and here applied to solid-phase−vapor-phase transitions and to a kinetic conglomerate. The exceptional accuracy of the rule is striking: sublimation of Ala, Leu, Pro, or Val gave almost a double amount of the sublimed material in comparison to the sublimation of each enantiopure (see the Supporting Information, Table S2). These experimental results undoubtedly support the independent saturation of the vapor phase by each enantiomer and the behavior of kinetic conglomerate of mixtures of enantiomers for these four amino acids.

The only aromatic amino acid studied by us, namely phenylalanine, gave different results (Figure 1, black curve). A possible kinetic conglomerate behavior could be proposed to explain more or less the left part of the curve but is not observed on the right side. The temperature of the sublimation (140 °C) being considerably higher than those used for other amino acids, this could have promoted "D + L into DL form transformation" (at least partially) and could explain the curve and the small plateau of close ee values among sublimates obtained starting from 50−80% ee mixtures (see below). This hypothesis is supported by the striking decrease of the weight obtained in the sublimation of mixtures of enantiomers of Phe after 3 h in comparison to the sum obtained from each enantiomer (see Table S2, Supporting Information).

In the case of L + DL mixtures, the samples were solubilized and then dried and ground before sublimation. The results are reported in Figure 2 (see also the Supporting Information, Table S3). Some experiments were performed with D + DL mixtures, giving ee values with data comparable to those obtained starting from L + DL mixtures (Table S3). Here, the most remarkable difference from the previous experiments (Figure 1) is that starting from mixtures with low ee the sublimates are enantioenriched but depletion takes place for the sublimates coming from mixtures of high ee. For leucine, phenylalanine, and proline there are distinct plateaus of close values and, regardless of the ee of the starting mixtures, the ee values of the sublimates lie in the ranges 75−90% (Phe), 42−52% (Leu), and 34−46% (Pro) ee. At the opposite, such a plateau is not observed for alanine and valine.

Several possible behaviors can be proposed.

(i) The sublimation of monomers occurs under thermodynamic control, leading to the formation of a gaseous phase with a euatmotic composition.[7,8,16]

(ii) The sublimation of each enantiomer is controlled by its saturated vapor pressure (kinetic conglomerate).

(iii) The vapor phase of each component is never saturated and a more or less statistical sublimation is observed: the ee of the sublimate is more or less that of the starting mixture. However, four and five cases can be proposed when dimeric or oligomeric compounds are present in the gas phase.

(iv) Independent sublimation of the racemic compound and pure enantiomer at different rates causes a change in the enantiomeric purity[5,12]—in this case we need dimers or oligomers in the gas phase to explain why molecules of an enantiomer coming from DL crystals do not interact with those coming from an enantiopure species.

(v) The formation of clusters occurs in the gas phase. It has been quite recently reported that natural α-amino acids are not only present as monomers in the gas phase: depending on the nature of the amino acid, the number of molecules in noncovalent oligomeric clusters can reach up to 12 units.[13,18]

For Leu, Pro, and Phe, the formation of a euatmotic mixture in the gas phase (behavior i) reasonably corresponds to the obtained data and indicates a thermodynamic control under our experimental conditions. In this case, a constant composition of the gas phase should be observed for the ee independently of the starting mixture except for 0 and 100%; near these two data, the results characterize the contribution of nonthermodynamic factors.

Considering the shape of the curves for “DL + L” Ala and Val mixtures (Figure 2, marked in red and blue, respectively), a logical deduction can be made that for these amino acids some intermediate case describes what is going on. The complete absence of any kind of plateau for Ala and Val argues a superposition of two or more behaviors. Behavior iv above has already been checked in the case of leucine using two sublimators, one containing an enantiopure species and the other the racemate.[14] Both sublimates were mixed at the end and an ee value comparable to that obtained using only one sublimator was found in several experiments. However, similar experiments cautiously performed on valine, proline, and alanine gave different results (see the Supporting Information, Table S4) and makes doubtful the hypothesis (iv) of independent sublimation of racemic and enantiopure species using only one sublimator. Thus, we can just ascribe the sublimation of Ala and Val to the lack of a well-defined process controlled by the vapor pressure of an identified mixture or of each component.

Can we extrapolate such behaviors to more complex mixtures? Can we observe synergistic effects? Several binary and ternary mixtures involving amino acids of comparable volatility (Ala, Val, Leu) were partially sublimed under our experimental conditions (Table 1). Although there are some striking differences from monocomponent nonracemic mixtures, it is difficult to deduce rules from these five experiments. Thus, there is a considerably higher enantioenrichment for the binary mixture (entry 1) in comparison to that starting from Ala and Leu 10% ee simple mixtures. However, starting from 70% ee, an increase of ee for Leu is observed while the ee of Ala decreases (entry 2). For entries 3 and 5, the results are not far from those obtained with each amino acid, but for the fourth entry, enrichment lower than that obtained for the individual nonracemic mixture of Leu was observed.

Table 1. Sublimation of Binary and Ternary Mixtures of Amino Acids[a]

entry	starting mixture,[b] % ee	sublimate, % ee
1	10 (L-Ala), 10 (L-Leu)	41.2 (L-Ala), 61.3 (L-Leu)
2	70 (L-Ala), 70 (L-Leu)	60.4 (L-Ala), 78.4 (L-Leu)
3	70 (L-Ala), 10 (L-Leu)	63.7 (L-Ala), 46.1 (L-Leu)
4	10 (L-Ala), 10 (L-Leu), 10 (L-Val)	13.2 (L-Ala), 20.9 (L-Leu), 17.3 (L-Val)
5	70 (L-Ala), 70 (L-Leu), 70 (L-Val)	55.9 (L-Ala), 67.2 (L-Leu), 62.9 (L-Val)

[a]Conditions: 14 h, 95−100 °C. [b]Equal amounts of amino acids in all cases.

Since all the mixtures were prepared by presolubilization and evaporation of water under vacuum, the mutual absorption or occlusion within crystal lattices should not be excluded,[19] but the physical properties of the thus formed cocrystals have never been studied. However, the hypothesis of a huge enrichment of all the ee’s by partial sublimation of complex mixtures of amino acids[12] is not observed here.

■ CONCLUSION

In conclusion, at the lowest temperature of sublimation, in order to be as near as possible to an equilibrium, the sublimate of an enantioenriched amino acid presents properties which are characteristic of this amino acid but which cannot be rationalized for all of them by a euatmotic composition of the gaseous phase, as observed for many other compounds. This particular behavior of amino acids could find its origin in the required energy to transform the zwitterionic form observed in the solid state to monomeric nonionic species detected in the gas phase or/and to the presence of dimers, oligomers, or clusters in the gas phase.[18] Therefore, the thermodynamic euatmotic equilibrium for amino acids needs a long time to be form or, for other cases, is a theoretical state which has no sense for mixtures containing not only monomeric structures.

Such behaviors open the way to many studies under various conditions more or less distant from the equilibrium to find new ways for the separation of enantiomers. These and our parallel studies on phase transitions[20] also give information related to the origin of homochirogenesis on the Primitive Earth showing that, under nondrastic conditions, the partial sublimation of enantioenriched amino acids can explain an increase of the ee of a sublimate or residue but such an increase remains constrained in the range by the physical properties of each component.

■ EXPERIMENTAL SECTION

The mixtures of amino acids were prepared by weighing the components on analytical balances accurate up to 10^{-4} g. Before sublimation “L + DL” and multicomponent mixtures (1 g) were dissolved in distilled water with heating (25 mL for Ala and Val; 60 mL for Leu). The mixtures of Phe were prepared by mechanical mixing.

The resulting solutions were cooled to room temperature and evaporated using a rotary evaporator at 40 °C. The residues were dried in vacuo (0.1 mbar) with a hot water bath over several hours (2−3 h). The powder was carefully ground with a mortar and a pestle and transferred to the bottom of a sublimator through a long-nozzle funnel, avoiding the presence of powder on the walls. The sublimations were conducted over 6−14 h at a controlled temperature and pressure (0.1 mbar). The sublimate was collected from the cold finger by washing with hydrochloric acid (~10 mL, 1 M). The solvent was removed using a rotary evaporator and dried in vacuo. The residue (hydrochloride) was weighed and derivatized using ethyl chloroformate under basic conditions[21] for analysis by chiral GC (GC capillary CHIRALDEX G-TA column (length × i.d. 30 m × 0.25 mm, d_f (film thickness) = 0.12 μm) on a Shimadzu GC-2014 instrument). The mass of the free amino acid was calculated from the mass of the hydrochloride.

Concerning the preparation of “L+D” samples, the enantiomers were crystallized separately in the same amount of solvent, dried, weighed, and carefully mixed.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Text giving the parameters of chiral GC analysis, three tables giving the enantiomeric excesses of sublimates, and one table giving the weights. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail for J.-C.G.: jean-claude.guillemin@ensc-rennes.fr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge the financial support of the PHC DNIPRO 2013 project (Nr. 28318QA and M/7-2013) and the State Agency on Science, Innovation and Informatization of Ukraine. J.-C.G. thanks the Centre National d'Etudes Spatiales (CNES) for financial support.

■ REFERENCES

(1) Most authors (including in our previous works) have used the term "eutectic" for the equilibrium points of all phase diagrams. According to IUPAC recommendations, for the "solid−gas" transition the term "euatmotic" ought to be used and for the "solid−solution" phase diagrams "eutonic". (a) Clark, J. B.; Hastie, J. W.; Kihlborg, L. H. E.; Metselaar, R.; Thackeray, M. M. *Pure Appl. Chem.* **1994**, *66*, 577−594. (b) Gamsjäger, H.; Lorimer, J. W.; Scharlin, P.; Shaw, D. G. *Pure Appl. Chem.* **2008**, *80*, 233−276 and references therein.

(2) Cintas, P. *Angew. Chem., Int. Ed.* **2008**, *47*, 2918−2920.

(3) Han, J.; Nelson, D. J.; Sorochinsky, A. E.; Soloshonok, V. A. *Curr. Org. Synth.* **2011**, *8*, 310−317.

(4) Garin, D. L.; Greco, D. J. C.; Kelley, L. *J. Org. Chem.* **1977**, *42*, 1249−1251.

(5) Soloshonok, V. A.; Ueki, H.; Yasumoto, M.; Mekala, S.; Hirschi, J. S.; Singleton, D. A. *J. Am. Chem. Soc.* **2007**, *129*, 12112−12113.

(6) Bellec, A.; Guillemin, J.-C. *J. Fluorine Chem.* **2010**, *131*, 545−548.

(7) Jacques, J.; Collet, A.; Wilen, S. H. *Enantiomers, Racemates and Resolutions*; Wiley: New York, 1981; pp 159−165.

(8) Eliel, E. L.; Wilen, S. H.; Mander, L. N. *Stereochemistry of Organic Compounds*; Wiley: New York, 1994; pp 179−183.

(9) Farina, M. *Chem. Commun.* **1987**, 1121−1122. Farina, M.; Silvestro, G. D. *Mol. Cryst. Liq. Cryst.* **1988**, *161*, 177−198.

(10) Bellec, A. *Ph.D. Thesis*, University of Rennes, 2009; No. 4010.

(11) Albrecht, M.; Soloshonok, V. A.; Schrader, L.; Yasumoto, M.; Suhm, M. A. *J. Fluorine Chem.* **2010**, *131*, 495−504.

(12) Fletcher, S. P.; Jagt, R. B. C.; Feringa, B. L. *Chem. Commun.* **2007**, 2578−2580.

(13) Perry, R. H.; Wu, C.; Nefliu, M.; Cooks, R. G. *Chem. Commun.* **2007**, 1071−1073.

(14) Bellec, A.; Guillemin, J.-C. *Chem. Commun.* **2010**, *46*, 1482−1484.

(15) An assumption was made that the composition at the euatmotic points should be equal or close to that at the eutonic points measured for aqueous solutions.[16] However, this assumption is erroneous: striking differences simply by changing the solvent or even "tuning" by the addition of achiral substances were observed. Klussmann, M.; Izumi, T.; White, A. J. P.; Armstrong, A.; Blackmond, D. G. *J. Am. Chem. Soc.* **2007**, *129*, 7657−7660. To the best of our knowledge, the euatmotic points of amino acids, if they exist, have never been determined to date, which can be easily explained by their very low vapor pressure.

(16) Blackmond, D. G.; Klussmann, M. *Chem. Commun.* **2007**, 3990−3996.

(17) Meyerhoffer, W. *Ber. Bunsen.-Ges. Phys. Chem.* **1904**, *37*, 2604−2610.

(18) Yang, P.; Xu, R.; Nanita, S. C.; Cooks, R. G. *J. Am. Chem. Soc.* **2006**, *128*, 17074−17086.

(19) Weissbuch, I.; Lahav, M. *Chem. Rev.* **2011**, *111*, 3236−3267.

(20) Tarasevych, A. V.; Sorochinsky, A. E.; Kukhar, V. P.; Guillemin, J.-C. *Origins Life Evol. Biospheres* **2013**, *43*, 129−135.

(21) Hušek, P. *J. Chromatogr.* **1991**, *552*, 289−299.