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DETERMINATION OF THE ENHANCEMENT OF THE ENANTIOMERIC PURITY DURING RECRYSTALLIZATION OF AMINO ACIDS

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SUMMARY

The enantiomeric purity of commercially available amino acids has been determined down to 0.01% before and after recrystallization. The amino acids have been transformed smoothly into the volatile N-trifluoroacetyl amino acid methyl esters, and were investigated by gas chromatography on the chiral stationary phase Chirasil-Val.

INTRODUCTION

The determination of the stereochemical integrity of chiral compounds is a major analytical task for both synthetic organic chemistry and life science. Among different spectroscopic and chromatographic techniques[1,2], gas chromatography (GC) on chiral stationary phases[3–7] such as L or D-Chirasil-Val[8,9] is the most promising approach for the determination of exceedingly high enantiomeric purities[9,10].

In order to calculate the degree of inversion in diazotization reactions of amino acids, the enantiomeric purity of both the starting material and the final product has been determined[11–13]. In peptide synthesis, the degree of racemization has been estimated after hydrolysis[14,15]. However, the enantiomeric purity of the starting amino acids, a well as of any derivatives prepared thereof, is still a matter of debate. In the present study, we report on the enantiomeric purity of several batches of amino acids from commercial sources, and on the further enrichment of the major enantiomer by a simple recrystallization procedure.

EXPERIMENTAL

Materials

L-Amino acids were kindly provided by Merck (Darmstadt, F.R.G.), Fährhaus Pharma (Hamburg, F.R.G.) and Ajinomoto (Tokyo, Japan), D-amino acids by Degussa (Hanau, F.R.G.). L-Serine was obtained from Medac (Hamburg, F.R.G.).

Recrystallization of the amino acids

Typically, a sample of 100 g of the amino acid was dissolved partially in water and then stirred under reflux. Within 5 h, water was added until the amino acid was

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completely dissolved. The solution was allowed to cool to ambient temperature and was then kept at 4°C for several hours. The crystals of the amino acid were filtered off, rinsed with small portions of water and dried carefully.

Alternatively, procedure[16], using water–ethanol (9:1, v/v) as the solvent was employed. Thus, the amino acid was dissolved in water at 50°C, the necessary volume of ethanol was added, the solution was allowed to cool to ambient temperature and was then kept at 4°C for several hours. The crystals were rinsed carefully with water and dried.

Standard procedure for the derivatization of amino acids

A sample of the recrystallized amino acid was dissolved in a solution of hydrogen chloride in methanol, prepared by mixing acetyl chloride and methanol, 1:10 (v/v). A 0.5-ml volume of the reagent was used per derivatization reaction, carried out in a 1-ml Reactivial (Macherey-Nagel, Düren, F.R.G.) and heated for 10 min at 110°C. The solvent was removed completely in a stream of dry nitrogen. The residue was allowed to react with trifluoroacetic anhydride (100 μl) in dichloromethane (500 μl) for 10 min at 110°C. The reagent was carefully evaporated in a stream of dry nitrogen, in order completely to remove the by-product trifluoroacetic acid. The derivative should cover the surface of the Reactivial as a thin film. Eventually, the residue was dissolved in 0.1–0.5 ml dichloromethane.

Gas chromatography

GC was performed with a Carlo Erba Fractovap 2102 or 2150, equipped with a laboratory computer Trivector Trilab II. Fused-silica capillary columns (25 m × 0.3 mm) were coated with Chirasil-Val, as described[17].

RESULTS AND DISCUSSION

While many racemic compounds form a conglomerate, *i.e.*, a mixture of crystals containing either one of the enantiomers, mixed crystals are often encountered[18]. In preparative organic chemistry the enhancement of the enantiomeric excess (e.e.) of enantiomerically enriched mixtures by the formation and recrystallization of a suitable derivative is a technique often applied, and occasionally well documented, *e.g.*, via the calorimetrically determined melting curve or by NMR spectroscopy after diastereomer formation[19].

When we started to monitor by GC the enantiomeric enrichment of amino acids during recrystallization our attention was primarily focused on alanine, for various reasons. First, the trifluoroacetyl derivative N-TFA-Ala-OCH_3 is formed in a smooth and rapid reaction sequence. Secondly, the chromatographic properties on Chirasil-Val are particularly well suited. The capacity factors k', are small, and the resolution factor, α, is fairly high, thus enabling rapid and accurate analysis of the enantiomeric composition. Last not least, some physical properties of particular interest are documented in the literature. While the solubilities of one antipode and the racemate in water differ only slightly, dependent on the temperature[20], as shown in Fig. 1, there are pronounced differences in the crystalline state[21,22]. In the L-form, a particular molecule, depicted by solid lines in Fig. 2a, is obviously surrounded by molecules with L-configuration, only. In the DL-form, the environment of a particular molecule with

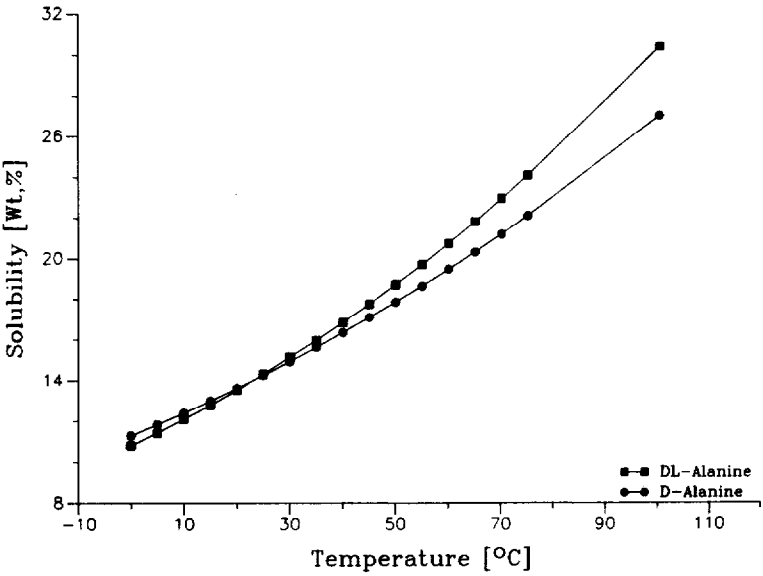

Fig. 1. Solubility of D-alanine and DL-alanine in water as a function of temperature. Values taken from ref. 20.

L-configuration, depicted by solid lines in Fig. 2b, is composed of L- (shaded) and D-stereoisomers. The intermolecular interactions differ accordingly, which is also seen in the hydrogen bonding network. With respect to the recrystallization of L-alanine containing only traces of the D-antipode, Fig. 2a is more relevant. The question is to what extent a molecule with L-configuration can be erroneously replaced by one with D-configuration, apart from the possibility of non-stereospecific growth on a particular face of the crystal[23].

Therefore, the alanine crystals were washed thoroughly with water. The derivatization to N-TFA-Ala-OCH_3 was performed under standard conditions, *i.e.*, esterification with HCl in methanol at 110°C for 10 min, and amide formation with trifluoroacetic anhydride in dichloromethane at 110°C for 10 min. It has been shown that racemization is only significant at higher temperatures[24].

Samples containing the L-antipode in excess were measured on capillaries coated with L-Chirasil-Val, and samples with D in excess on D-Chirasil-Val, in order to make sure that the minor peak is eluted in front of the major one[9]. The data obtained for L- and D-alanine are compiled in Table I. The relative standard deviation was less than 10% of the percentage of the minor enantiomer. The data for D-alanine deserve a comment. In order to circumvent the variability in the enantiomeric purities of different crystals, an average value was determined. Thus, the original crystals were dissolved completely, an aliquot of the solution was taken and dried in a stream of nitrogen, prior to derivatization. The same procedure was followed for crystal fractions 1 and 2. The enhancement in e.e. during recrystallization is evident. The values are found to vary slightly from one crystal to another, and even a lowering in e.e. was observed in the third recrystallization step when looking at one of the crystals. Hence

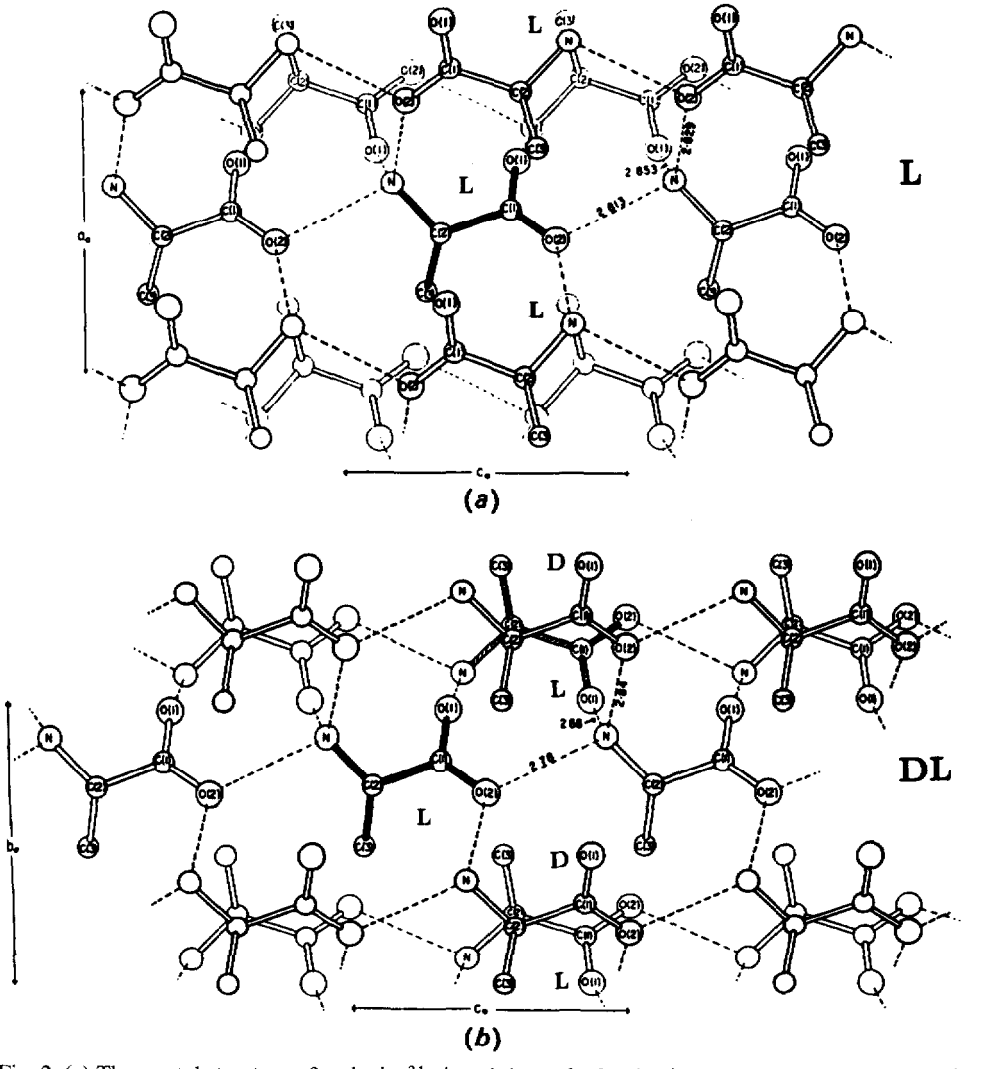

Fig. 2. (a) The crystal structure of L-alanine[21] viewed down the *b* axis. (b) The structure of DL-alanine[22] viewed down the *a* axis.

it is highly recommended that any studies on the enantiomeric purity of solid compounds be performed on an aliquot of the completely dissolved material. The chromatograms for D-alanine are shown in Fig. 3.

Rechromatography of the sampled rough data, followed by checking the y-expanded chromatograms on the screen of the laboratory computer Trilab II, proved to be a useful technique to get reliable data. Where the integrating program could not cope satisfactorily with the rough data, the chromatograms were plotted twice, using different y-factors, and xeroxed three-fold. The peaks were cut and weighed.

Typical average values for some amino acids are listed in Table II, before and

TABLE I

ENANTIOMERIC PURITIES (e.e.) OF ALANINE BEFORE AND AFTER RECRYSTALLIZATION FROM WATER, AS DETERMINED BY GC OF N-TFA-ALA-OCH_3 ON CAPILLARY COLUMNS COATED WITH L- AND D-CHIRASIL-VAL, RESPECTIVELY

Sample	*% of minor antipode before and after recrystallization*	*% e.e.*
L-Ala	0.23	99.54
L-Ala 1st crystals	0.022	99.956
L-Ala 2nd crystals	0.019	99.962
D-Ala	1.75	96.50
D-Ala 1st crystals	0.098	99.804
D-Ala 2nd crystals	0.039	99.922
D-Ala 3rd crystals	0.066	99.868

after recrystallization. The relative standard deviation was less than 10% of the percentage of the minor enantiomer. In all cases investigated, a significant enhancement in e.e. was observed. A chromatogram for one of the recrystallized samples of methionine is shown in Fig. 4.

It is interesting that a commercial sample of L-serine contained an unacceptable high percentage of the D-antipode, *i.e.*, more than 1.2. One should be aware of the fact that all commercial compounds are crystalline samples, in other words, they had already undergone at least one recrystallization step. The surprisingly low e.e. value

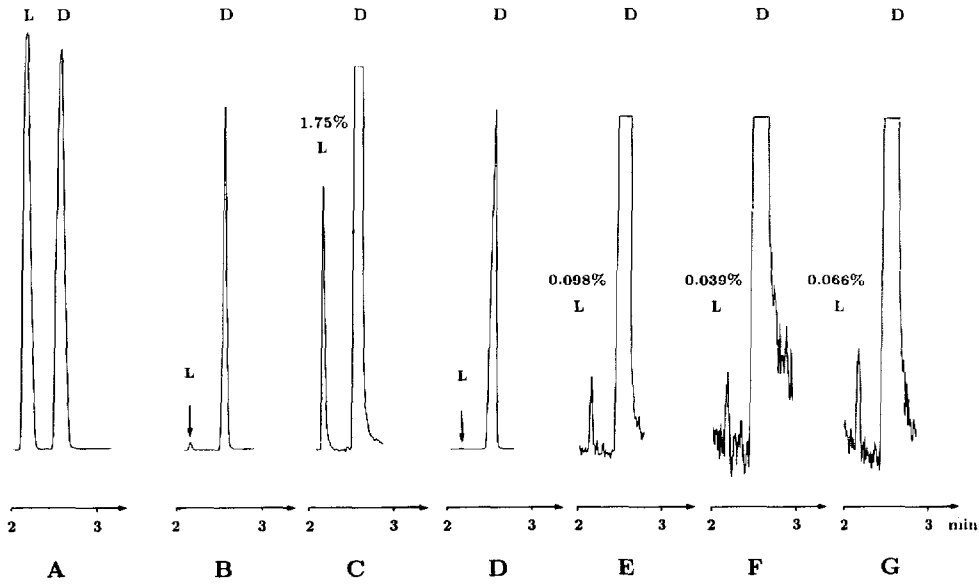

Fig. 3. Enantiomeric purity of alanine, determined N-TFA-Ala-OCH_3 on D-Chirasil-Val (H41, fused-silica capillary column, 25 m × 0.3 mm) at 70°C, 0.4 bar H_2, flame ionization detection (FID). (A) racemic alanine; (B) commercial sample of D-alanine, in scale; (C) expanded; (D) crystals from recrystallization 1; (E) expanded; (F) crystals from recrystallization 2; (G) one crystal from recrystallization 3.

TABLE II

ENANTIOMERIC PURITIES (e.e.) OF L-AMINO ACIDS BEFORE AND AFTER RECRYSTALLIZATION FROM ETHANOL–WATER (1:9), AS DETERMINED BY GC ON A CAPILLARY COLUMN COATED WITH L-CHIRASIL-VAL

Amino acid	*%D before and after recrystallization*		*% e.e. after recrystallization*
L-Val	0.05	0.017	99.966
L-Leu	0.07	0.040	99.920
L-Phe	0.15	0.018	99.964
L-Met	0.17	0.094	99.812
L-Thr	0.25	0.009	99.982
L-Asp	0.42	0.032	99.936
L-Ser	1.26	0.675	98.650

of L-serine, as compared to the rather similar amino acid L-threonine, can be at least partly explained by the low efficiency of the recrystallization step. The crystals thus formed contained still more than 0.67% D.

However, even for alanine which usually shows good enhancement characteristics, an unacceptable high D content of up to 2.5% was occasionally found in commercial samples. We therefore strongly recommend that the enantiomeric purity of commercial batches be checked carefully, prior to any synthesis.

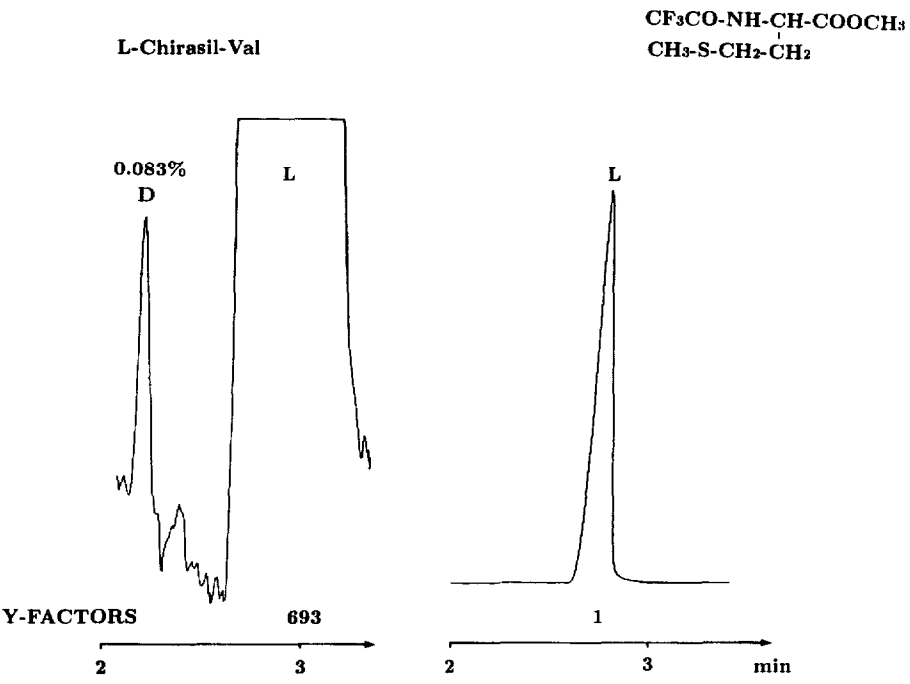

Fig. 4. Enantiomeric purity of one of the recrystallized samples of L-methionine, determined as N-TFA-Met-OCH_3 on L-Chirasil-Val (duran glass capillary column, 20 m × 0.25 mm) at 120°C, 0.4 bar H_2, FID. Right hand: in scale; left hand; expanded in *y*-direction.

CONCLUSIONS

Due to their exceedingly high enantiomeric excess (e.e.), recrystallized amino acids are a useful tool to pinpoint the possible degree of racemization during derivatization under standard conditions. Besides this, they are both a challenge and a measure for the sensitivity and reliability of the state of the art of quantitative GC on capillary columns. In view of the increasing demand for biologically active compounds of high e.e., the enhancement of the enantiomeric purity by recrystallization of solid starting materials should be seriously considered.

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