

ENANTIOMERIC RESOLUTION OF α,α -DIALKYL- α -AMINO ACIDS: SURVEY ON CHROMATOGRAPHIC METHODS AND RELEVANCE FOR ASTROBIOLOGY

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Introduction: The formal substitution of the C $^{\alpha}$ -hydrogen atoms of glycine by alkyl groups R^{1,2} yields α,α -dialkyl- α -amino acids (AAAs) of the general structure H₂NC(R¹,R²)COOH. If R¹ differs from R² the resulting amino acids are chiral. The structurally simplest achiral AAA in this group is α -aminoisobutyric acid (α -AIB) and the simplest chiral AAA is isovaline (Iva). In some carbonaceous meteorites considerable L-enantiomeric excesses were found in Iva and other chiral AAAs despite their commonly accepted abiotic origin [1, 2]. However, sequences of a rapidly increasing number of fungal polypeptide antibiotics (peptaibols/peptaibiotics) are reported containing high proportions of α -AIB together with D- or L-Iva or even both enantiomers in the peptides [3, 4].

Results and Discussion: Here, we report on the enantiomeric resolution of a series of AAAs (R¹ = methyl, R² = ethyl to nonyl) with emphasis on the separation of DL-Iva. For the (indirect) enantiomeric resolution we used pre-column derivatization with *o*-phthalaldehyde (OPA) together with chiral thiols (Boc-L-cysteine, *N*-Ac-L-cysteine, *N*-isobutyryl-L-cysteine) or derivatization with Marfey's reagent (5-fluoro-2,4-dinitrophenyl-L-alanine amide). The resulting diastereoisomers were resolved by RP-HPLC. For the direct enantioresolution AAAs were converted into *N*-perfluoroacyl-AAA-alkyl esters and analyzed by GC on chiral capillary columns available as Chirasil-L-Val and Lipodex E. Derivatization with OPA/chiral thiols afforded acceptable resolutions of DL-AAAs. Much better resolutions were obtained by derivatization with original Marfey's reagent or chiral variants developed in our laboratories [5]. Using GC best resolutions were obtained on Lipodex E.

Application of the methods to fungal extracts revealed the production of biotic α -AIB and D- or L-Iva by many taxa of hyphomycetous fungi. Consequently, α -AIB and Iva are considered as being fairly common in the terrestrial biosphere [3]. These findings are of relevance for astrobiology provided AAAs are detected in extraterrestrial environments [6, 7]. Furthermore, although there is little doubt that the AAAs occurring in carbonaceous meteorites are of non-biotic origin, there is a need for reliable methods to distinguish between biotically and abiotically synthesized AAAs (see abstract by J.E. Elsila et al., this volume).

References: [1] Pizzarello S., Cronin J.R. (2000) *Geochim Cosmochim Acta* 64:329-338. [2] Glavin D.P., Dworkin J.P. (2009) *PNAS USA* 106:5487-5492. [3] Degenkolb T. et al. (2007) *Chem Biodivers* 4:1052-1067. [4] De Zotti M. et al. (2010) *Chem Eur J.* 16:316-327. [5] Bhushan R., Brückner H. (2004) *Amino Acids* 27:231-247. [6] Strasdeit H. et al. (2001) *Chem Eur J* 7:1133-1142. [7] Thiemann W. H.-P., Meierhenrich U. (2001) *Orig Life Evol Biosph* 31:199-210.