5. Conclusion

In this contribution, we have presented some of the first successful results of the combination CZE-ISE. With its low detection limits for various cations, the ISE is a powerful sensor for charged species. Due to its high internal resistance, this sensor is almost perfectly suitable as a detector in zone electrophoresis. To fully exploit the analytical possibilities of this method, efforts are in progress to extend this technique to anions and organic cations.

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Fig. 10. Free zone electropherogram of doubly distilled water stored in a washbottle. Capillary I.D. 25 μm; length 0.99 m; buffer 20 mm magnesium acetate (HC2O); injection electrokinetically, 5000 V for 5 s; potential 20 kV; ISE membrane as described previously; detection post-column.

(S)-Trolox™ Methyl Ether: a Powerful Derivatizing Reagent for the GC Determination of the Enantiomers of Aliphatic Alcohols

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Abstract. (S)-Trolox™ methyl ether 5a prepared from the racemic commercial antioxidant Trolox™ is presented as a new chiral reagent for the GC analysis of stereoisomeric primary and secondary alcohols. The superiority to the hitherto known reagents is demonstrated by some examples.

1. Introduction

New methods of stereoselective organic synthesis, particularly some involving homogeneous asymmetric catalysis \cite{1}, yield products with high enantiomeric excess. For the development and the improvement of such techniques, reliable analytical procedures for ee determinations are essential. In the course of our work aimed at the total synthesis of naturally occurring tocopherols I and other isoprenoids, we realized that general methods for the exact determination of the (high) optical purity of intermediates comprising Me-branched C–C chains were lacking. Thus, we developed a practical GC method for the stereochemical analysis of acyclic terpenoid carbonyl compounds using acetics derived from (±)-1,1-tartaric esters \cite{2}.

In the case of Me-branched primary alcohols, we could obtain no satisfactory result by the application of commercially available chiral reagents. Recently, reports on the direct separation of a few such alcohols on GC columns coated with (chiral) cycloaliphatic derivatives have appeared \cite{3}. In this communication, we would like to offer a promising new derivatizing reagent for the analysis of such alcohols using conventional (achiral) GC columns.

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Secondary alcohols with the chiral center at the carbinol C-atom are much easier to separate than primary alcohols with Me-branched chains [12]. As expected from the above results, (S)-Trolox methyl ether Sa is an excellent reagent for such cases, sometimes leading to wide separations as shown for hexan-3-01 (2) (Fig., a).

In spite of the rapidly increasing potential of chiral stationary phases for chromatography [13], extension of the range of chiral reagents still appears warranted, as they offer generally useful methods for the separation of enantiomeric compounds on conventional high temperature columns. In addition, it is noteworthy that limitations of the GC method in the separation of diastereomeric esters derived from Sa (high molecular weight and thermal instability of the alcohol moiety) may be circumvented by the use of the more gentle conditions of capillary supercritical fluid chromatography (SFC) [14].

2. Results

Choosing racemic β-citronellol (3) as a candidate, we examined first the known chiral reagents [4][5]. In no case, we could achieve a satisfactory resolution. The work of Slover and Thompson [6], in which they have separated the four diastereoisomers of α-tocopherol (all-rac-1, R1 = R2 = Me) as the trimethylsilyl-ether derivative, constitutes a most remarkable separation of stereoisomers containing a Me-branched chain. Thus we decided to try a reagent with the structure of the chiral Me-substituted chromane moiety present in the natural isomer of α-tocopherol for the separation of 3.

The racemic carboxylic acid 5b/6b [7], commercially available [4a] as an antioxidant with the trade name Trolox™, gave us a facile access to such a reagent. Both enantiomers could be obtained by fractional crystallization (see Experimental) of the diastereoisomer salts 5c/6c with (−)-(S)-1-phenylethylamine in optically pure form (ee > 99.9%). Treatment with NaH/dimethyl sulfate and subsequent saponification of the methyl ester yield e.g. the (S)-enantiomer 5a [8]. To obtain 5a in gram quantities an alternative procedure has been worked out based on the chromatographic separation of glucosyl esters derived from 5a/6a [9].

As shown in the Figure (b), complete separation was achieved after esterification [10] of racemic citronellol (3) with (S)-Trolox methyl ether 5a. To investigate the scope of this new reagent, we applied it to a particularly difficult separation problem: (±)-2-methylpentan-1-ol (4) [3]. For comparison, the known reagents [4][5] were also applied. The analysis was carried out on an apolar (OV-1) capillary GC column [11].

The result demonstrates the limits of all these reagents: none of them achieved an analytically useful result, but in contrast to all the others, 5a showed at least a partial separation (Fig., c).
Monitoring of Carboxypeptidase Digestion by Matrix-Assisted Laser Desorption and Ionization Mass Spectrometry

Martin Schär*, K. Olaf Börnsten, Ernst Gassmann, and H. Michael Widmer

Abstract. The potential of matrix-assisted laser desorption and ionization mass spectrometry (LDI-MS) is demonstrated by monitoring and analyzing the digestion of (human) pTH (1–34), a synthetic peptide with carboxypeptidases Y and B. All occurring ion signals in the mass spectra could be identified as degraded peptides. By calculating the mass differences between successive degraded peptides, it was possible to identify the released amino acids and to determine the amino acids of the C-terminus of the original peptide. For a single MS measurement, only 2 pmol of substrate was needed. Time-course analysis of the cleavage of the first amino acid residue gave insight into the kinetics involved. These measurements strongly support the hope that quantitative information about concentrations can be extracted from LDI-MS.

1. Introduction

The determination of the amino-acid sequence in proteins is a major requirement in biomedical and biochemical research. With the stepwise Edman degradation of the N-terminus, this information can be obtained. This method is usually slow, it needs pure samples and can be impaired by chemical contaminations. Further N-terminal blocking groups can be a severe obstacle for the degradation process.

With the development of new mass spectrometric and ionization methods as fast-atom bombardment mass spectrometry (FAB-MS) [1], plasma-desorption mass spectrometry (PD-MS) [2][3], electrospray mass spectrometry (ES-MS) [4–6], and recently matrix-assisted laser desorption and ionization mass spectrometry (LDI-MS) [7–11], novel analytical tools have become available. They offer interesting opportunities for peptide sequencing in peptides and proteins; a major requirement in biomedical and biochemical research. With the stepwise Edman degradation of the N-terminus, this information can be obtained. This method is usually slow, it needs pure samples and can be impaired by chemical contaminations. Further N-terminal blocking groups can be a severe obstacle for the degradation process. With the development of new mass spectrometric and ionization methods as fast-atom bombardment mass spectrometry (FAB-MS) [1], plasma-desorption mass spectrometry (PD-MS) [2][3], electrospray mass spectrometry (ES-MS) [4–6], and recently matrix-assisted laser desorption and ionization mass spectrometry (LDI-MS) [7–11], novel analytical tools have become available. They offer interesting opportunities for peptide sequencing in peptides and proteins.