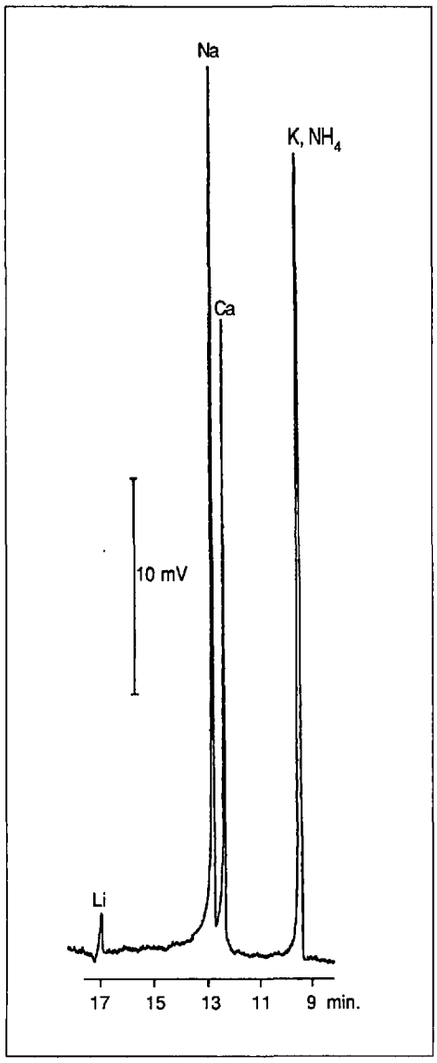
CHIMIA 45 (1991) Nr. 4 (April)



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 (HCl); injection electrokinetically, 5000 V for 5 s; potential 20 kV; ISE membrane as described previously; detection post-column.

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Chimia 45 (1991) 121–123

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

Willy Walther[1]*, Walter Vetter[1], Max Vecchi[1]†, Heinz Schneider[2], Robert Karl Müller[2], and Thomas Netscher[2]*

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 [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 **1** 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 acetals derived from (+)-L-tartaric esters [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) cyclodextrine derivatives have appeared [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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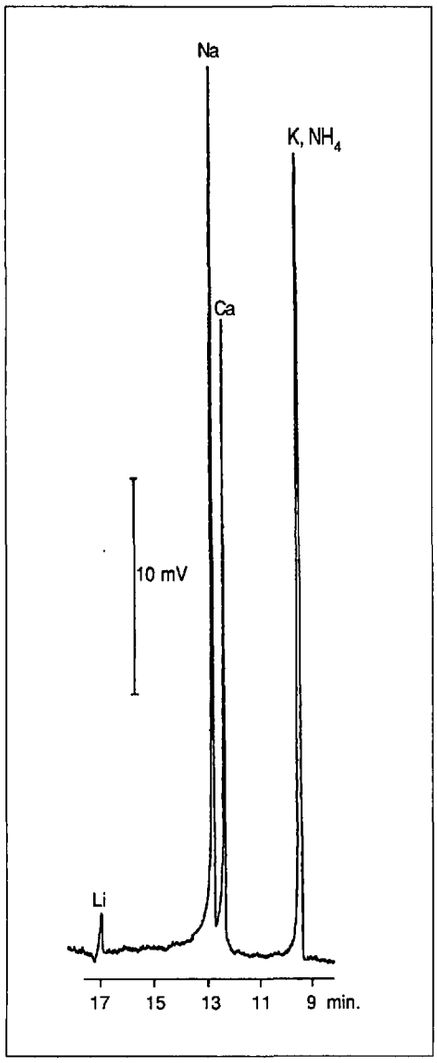

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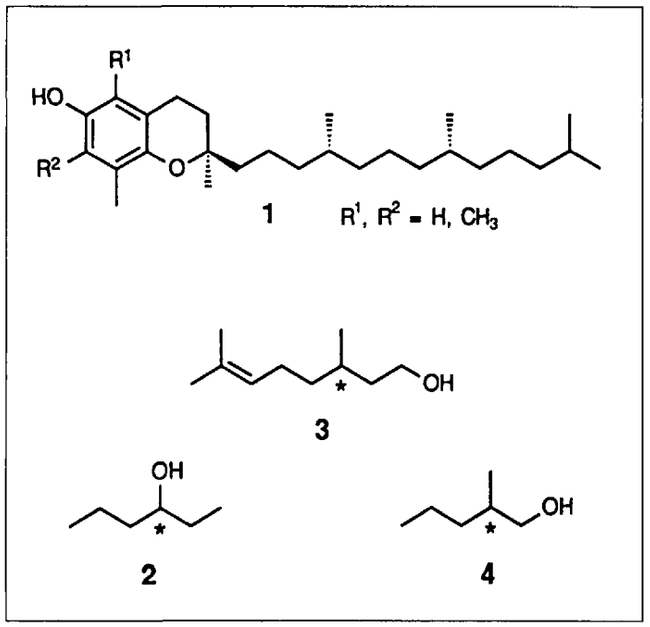

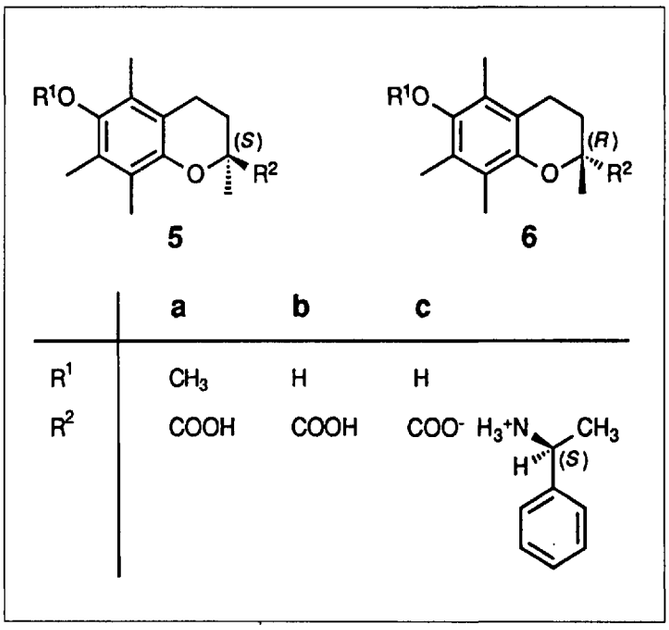

2. Results

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**, $R^1 = R^2 = Me$) 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 diastereoisomeric 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*).

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 **5a** is an excellent reagent for such cases, sometimes leading to wide separations as shown for hexan-3-ol (**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 diastereoisomeric esters derived from **5a** (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].

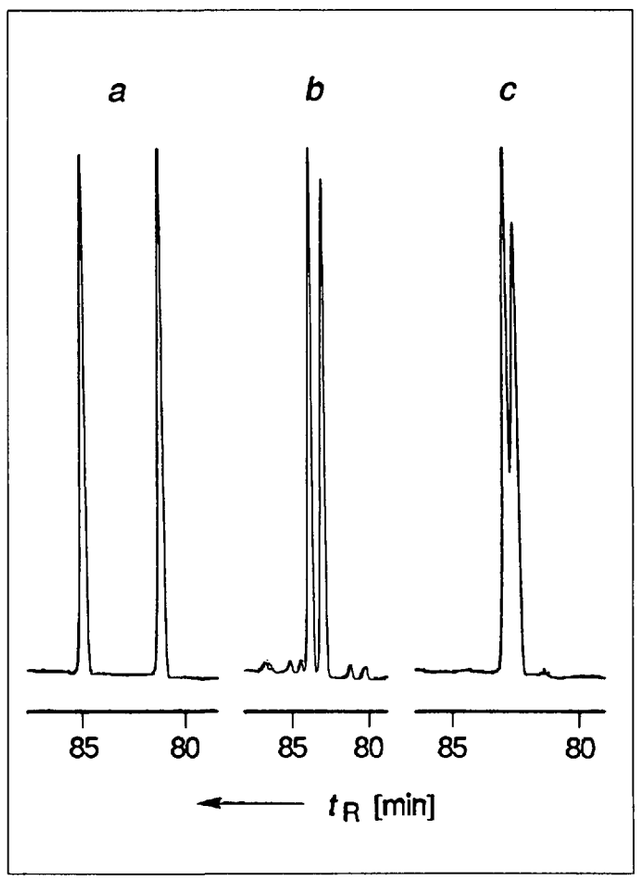

Figure. *Capillary gas chromatograms of the esters derived from* (S)-Trolox *methyl ether* **5a** *and the racemic alcohols hexan-3-ol* **2** (*a*), *β-citronellol* **3** (*b*), *and 2-methylpentan-1-ol* **4** (*c*).
Conditions: *OV-1* capillary column, 25 m, inner diameter 0.15 mm; carrier gas H_2, 50 cm/s; injector temp. 280°, detector temp. 280°; column temp. for *a*: 130–220°, *b*: 165–220°, *c*: 135–220° (temperature program with 0.5°/min).

We wish to thank Dr. *J.W. Scott* (*F. Hoffmann-La Roche Inc.*, Nutley, New Jersey) for providing us with a procedure for the resolution of *Trolox* and Ms. *K. Jakob* and Mr. *I. Gautschi* for technical assistance.

3. Experimental

(S)-3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic Acid ((S)-*Trolox*™, **5b**) *by Resolution.* (All glassware is cleaned by consecutive treatment with hot 10% NaOH soln., H_2O, and acetone prior to use.) To the soln. of 12.5 g (50.0 mmol) of *Trolox*™ in 20 ml of EtOH and 200 ml of Et_2O, filtered through a pad and stirred mechanically (*ca.* 250 rpm), are added (Ar atmosphere) 7.5 ml (59.0 mmol, 1.18 equiv.) of (–)-(*S*)-phenylethylamine in one portion. The temp. rises up to 27°, and, after stirring for 1 h, the *clear* pale yellow soln. (23°) is seeded by the addition of *ca.* 0.5 mg of **5c** [15]. Fine crystals begin to precipitate, and 3 h after addition of the amine, stirring is stopped. The mixture is allowed to stand at –20° for 21 h. Suction filtration and washing twice with Et_2O (40 ml each) yield, after drying *in vacuo*, 7.42–9.69 g of the ammonium salt containing *ca.* 77–94% of **5c** ($[\alpha]_{589}^{20}$ = –26 to –41; for all measurements: *c* = 1, EtOH). Isomerically pure **5c** [16] is obtained by twofold recrystallization (dissolving in 20 ml of hot EtOH, adding 200 ml of Et_2O and seeding): 5.89–6.50 g (32–35%) colourless crystals. M.p. (after sintering above 148°) 151–152°. $[\alpha]_{589}^{20}$ = –46.4.

For the hydrolysis, this salt is stirred in 100 ml of AcOEt and 50 ml of 2N HCl. After extraction with AcOEt, the org. layer is washed with H_2O (2 × 100 ml), dried ($MgSO_4$), and evaporated. Recrystallization from Et_2O/pentane (30/100 ml) and drying (0.2 mbar/100°) yield 3.40–4.03 g (27–32% overall from *rac*-**5b/6b**) colourless **5b**. M.p. = 161°. $[\alpha]_{589}^{20}$ = –67.1; ee > 99.9% (determined by GC analysis on a permethylated β-cyclodextrine phase after methylation by CH_2N_2 (reaction time 10 min, r.t.) and acetylation to the arylacetate-methylester).

(S)-3,4-Dihydro-6-methoxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-carboxylic Acid ((S)-*Trolox*™ *Methyl Ether*, **5a**). A soln. of 3.0 g (12.0 mmol) **5b** in 50 ml of THF is treated at r.t. successively with 2.5 equiv. each of NaH (1 h) and dimethyl sulfate (overnight). The crude oily methylether-methylester is saponified with 20.0 mmol of KOH in 25 ml of refluxing MeOH (19 h). Aq. workup and crystallization from Et_2O/hexane (5/15 ml, 0°) yield 89% of colourless **5a**. M.p. = 144–145°, $[\alpha]_{589}^{20}$ = –69.2; ee > 99.9% [8].

Esterification of Alcohols with **5a** [10]. To the soln. of *ca.* 15 μmol of alcohol in 1 ml of dry CH_2Cl_2 are added 10.5 mg (40 μmol) of **5a**, 15 mg (75 μmol) of 1,3-dicyclohexylcarbodiimide, and 0.1 mg (1 μmol) of 4-(dimethylamino)pyridine. The mixture is allowed to stand overnight at r.t. and filtered (*e.g.* by a syringe through a *Acrodisc CR PTFE* 0.45-μm filter; *Gelman Science Inc.*, USA). The filtrate is used for the GC analysis directly, or after washing with $NaHCO_3$ soln. and drying (Na_2SO_4).

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[4] Reagents used (suppliers: [a] *Fluka Chemie AG*, [b] *Janssen Pharmaceutica*, [c] *Sigma Chemical Co.*, [d] *Aldrich Chemical Co.*, [e] *IPS Chimie*): (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (*Mosher*'s acid)[a], (–)-camphanic acid [a], (–)-(menthyloxy)acetic acid [a], (+)-*trans*-chrysanthemic acid [b], (+)-(*S*)-2-(6-methoxy-2-naphthyl)propionic acid ((+)-*Naproxen*) [c], *N*-methyl-L-prolin [c], pentafluoropropionyl-L-prolin (from commercial L-prolin [a]), diacetone-2-keto-L-gulonic acid [a], (*S*)-2-[(phenylamino)carbonyloxy]propionic acid ((*S*)-PACOPA, *Brown*'s reagent) [b], (4*R*,5*R*)-2,3-*O*-isopropylidene-L-tartaric monoisopropylester [5], (4*R*,5*R*)-2-chloro-4,5-dimethyl-1,3,2-dioxaphospholan-2-oxide (*Anderson-Shapiro* reagent) [d], (*R*)-1,1'-binaphthyl-2,2'-diylphosphoric acid [a], (*R*)-1-phenylethylisocyanate [a], (*R*)-1-(1-naphthyl)ethylisocyanate [e].
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[14] For a successful example, see: S.R. Sumpter, in 'Analytical Supercritical Fluid Chromatography and Extraction', Eds. M.L. Lee and K.E. Markides, Chromatography Conferences, Inc., Provo, Utah, 1990, p. 543.
[15] Samples of **5c** are provided on request.
[16] From the combined mother liquors of all crystallizations, **6b** with low ee is obtained after acid hydrolysis. This can be converted as described (using the (*R*)-amine) to pure **6b/6a** in the same yield.

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Monitoring of Carboxypeptidase Digestion by Matrix-Assisted Laser Desorption and Ionization Mass Spectrometry

Martin Schär*, K. Olaf Börnsen, 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 8 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 peptides and 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

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