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Review

Cyclodextrin derivatives as chiral selectors for direct gas chromatographic separation of enantiomers in the essential oil, aroma and flavour fields

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Abstract

This article reviews papers published over the period 1995–1998 dealing with the application of cyclodextrin derivatives (CDs) as chiral selector for direct enantiomer GC separation of volatile optically active components in the essential oil, extract, flavour and aroma fields. For each application, the racemate analysed, the CD employed as chiral selector and the matrix investigated are reported. The applications are grouped by analytical technique employed: capillary gas chromatography and capillary gas chromatography–mass spectrometry (GC and GC–MS); two-dimensional gas chromatography (GC×GC); capillary gas chromatography–isotope ratio-mass spectrometry (GC–IRMS); liquid chromatography–capillary gas chromatography (LC–GC).

Keywords: Chiral selectors; Enantiomer separation; Reviews; Essential oils; Food analysis; Cyclodextrins

Contents

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1. Introduction

Derivatised cyclodextrins (CDs) are nowadays the most popular chiral selector in the direct GC enantiomer separation of volatile racemates. The use of CDs in routine enantiomer recognition is a well established and fast approach because the high enantioselectivity of CDs and the high stability and reproducibility of capillary GC columns prepared with CDs allow us to analyse a very large number of racemates reliably; moreover, more than 90% of racemates are separated without derivatisation.

Enantiomer separation and enantiomer excess (*ee*) or ratio (ER) determination is of great importance in the fields of flavours and essential oils (EOs), not only to characterise a vegetable matrix or extract or to evaluate the biosynthetic pathway of one or more of its components, but also to establish its origin and/or to identify possible adulterations.

A previous review [1] covered the articles that had appeared between 1989 and 1994, and considered interesting reviews by Mosandl [2,3] and Werkhoff et al. [4,5] who reviewed scope and limitations of enantioselective GC (Es-GC) and related techniques in this field, in particular, outlining trends and perspectives in origin and assessment of flavour and fragrance compounds.

This article reviews publications that appeared from June 1994 to June 1998 dealing with the separation of volatile racemates in essential oils and flavours by GC techniques using CDs as chiral selectors, and considers articles reported in *Chemical Abstracts* and/or the bibliography section of the *Journal of Chromatography, Gas and Liquid Chromatography Abstracts* and *Analytical Abstracts*. Because of the large number of publications in this field, we apologise in advance for any articles which may have been overlooked.

Theory, separation mechanisms, chromatographic behaviour, development of new CDs for GC or packed column GC separation will not be dealt with, and, only separations of underivatised compounds are considered.

As for the previous review [1], this article is divided into sections in function of the analytical technique adopted: capillary gas chromatography and capillary gas chromatography–mass spectrometry (GC and GC–MS); two-dimensional gas chromatography (GC×GC); capillary gas chromatography– and/or two-dimensional gas chromatography–isotope ratio-mass spectrometry (GC–IRMS; GC×GC–IRMS); liquid chromatography–capillary gas chromatography (LC–GC). Each section in turn consists of two subsections, the first dedicated to EOs and the second to extracts and flavour and aroma compounds: thus, the analysis of the same compound, either in the same or in different matrices may be reported in more than one section, if carried out with different techniques. For each reference, the racemate investigated, the CD adopted for the separation and the original matrix are reported; CDs are only reported with their chemical abbreviation or acronym; when not specified, OV-1701 (or OV-1701-OH, or OV-1701-*vi*) is the diluting phase; CD% is not indicated. A list of the abbreviations is reported in Section 7.

2. Capillary gas chromatography and capillary gas chromatography–mass spectrometry

2.1. Essential oils

The introduction of CDs as enantioselective stationary phases in GC has strongly contributed to popularising Es-GC in authenticity control of EOs. Koenig et al. [6] evaluated the potential of Es-GC with modified CD as chiral selectors for EO authenticity through the analysis of a selection of economically important EOs (*Melissa officinalis*, citronella, balm, *Eucaliptus citriodora*, bergamot, rose, geranium and mint EOs) and deduced that Es-GC is highly effective when enantiomerically pure constituents are present in natural oils, but inadequate in cases of (naturally) varying enantiomeric composition. Bicchi et al. [7] proposed exploiting the increased enantioselectivity of the most recent CDs, which have greatly enlarged the number of separable racemates, to obtain a reliable determination of origin and genuineness of an EO through crossed evaluation of the ER of several of its optically-active components, in a single GC run. They demonstrated the reliability of this approach through its applications to lavender, lemon, peppermint, rose and iris EOs. Fig. 1 shows the structures of the compounds in Figs. 2 and 3. Fig. 2 shows

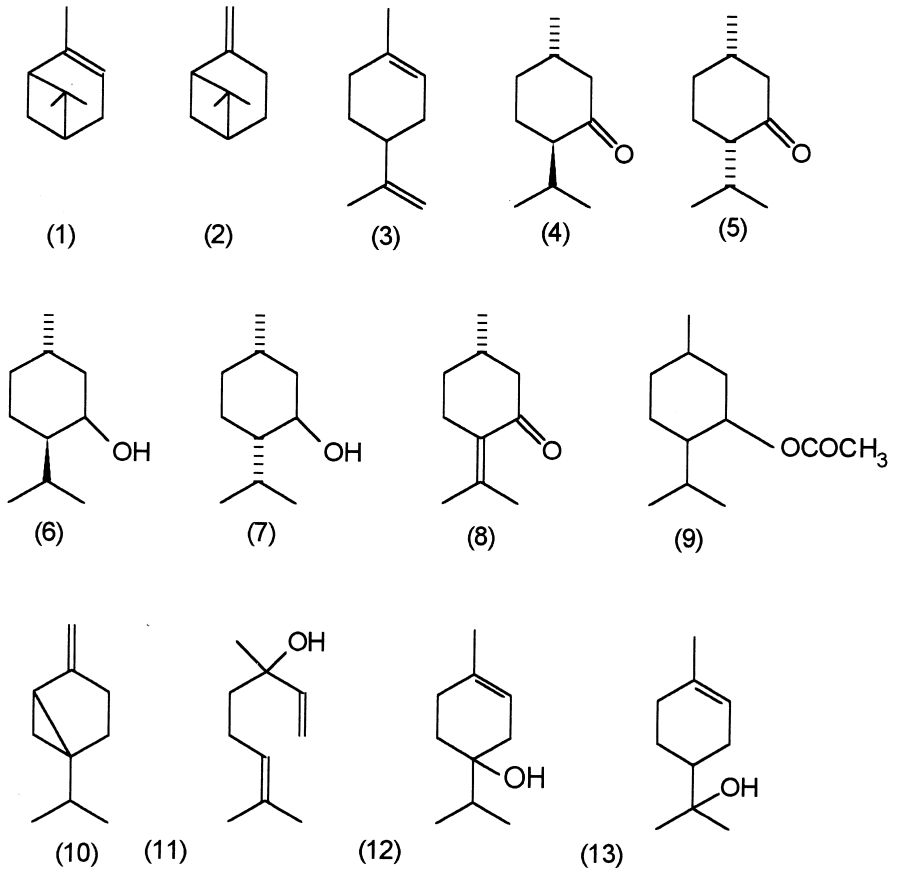

Fig. 1. Structures of the compounds in Figs. 2 and 3. 1: α-Pinene, 2: β-pinene, 3: limonene, 4: menthone, 5: isomenthone, 6: menthol, 7: isomenthol, 8: pulegone, 9: menthyl acetate, 10: sabinene, 11: linalol, 12: α-terpineol, 13: terpinen-4-ol.

Es-GC patterns of a peppermint EO and of its characteristic optically active components using 2,3-DiMe-TBS-β-CD as chiral selector.

Es-GC in combination with olfactometry has also given an important contribution to a correct determination of sensory properties and differentiation of individual enantiomers. With the aid of Chirbase/GC, Koppenhoefer et al. [8] combined configurations, odour characteristics of single enantiomers and GC properties of 17 important flavours and fragrances, 2-methylbutanoic acid, ethyl 2-methylbutanoate, 2-ethylhexanoic acid, 1-octen-3-ol, linalool, (*E*)-nerolidol, (*Z*)-nerolidol, limonene, α-ionone, α-terpineol, carvone, α-phellandrene, menthol, γ-decalactone, whiskey lactone, δ-decalactone, theaspirane and 2-methyl-4-propyl-1,3-oxathiane.

CD derivatives have also been demonstrated to be effective for isolation of pure enantiomers by preparative GC. Columns packed with CDs have widely been used for this purpose in particular by Koenig's group, who introduced this technique in this field and obtained up to milligram amounts of almost pure enantiomers, or at least mixtures enriched in one enantiomer [9]. Bicchi et al. [10,11] proposed using thick-film wide-bore (0.53 mm I.D.) columns for preparative capillary GC (Prep-cGC) of pure enantiomers, in particular when pure enantiomers must be isolated from complex mixtures. Since the loading capacity of thick-film wide-bore columns is low compared with packed columns, multiple injection in combination with cryo- or solvent accumulation is necessary to obtain amount of 1 or 2 mg of pure enantiomers. They also investigated the relationship between resolution values of two enantiomers on

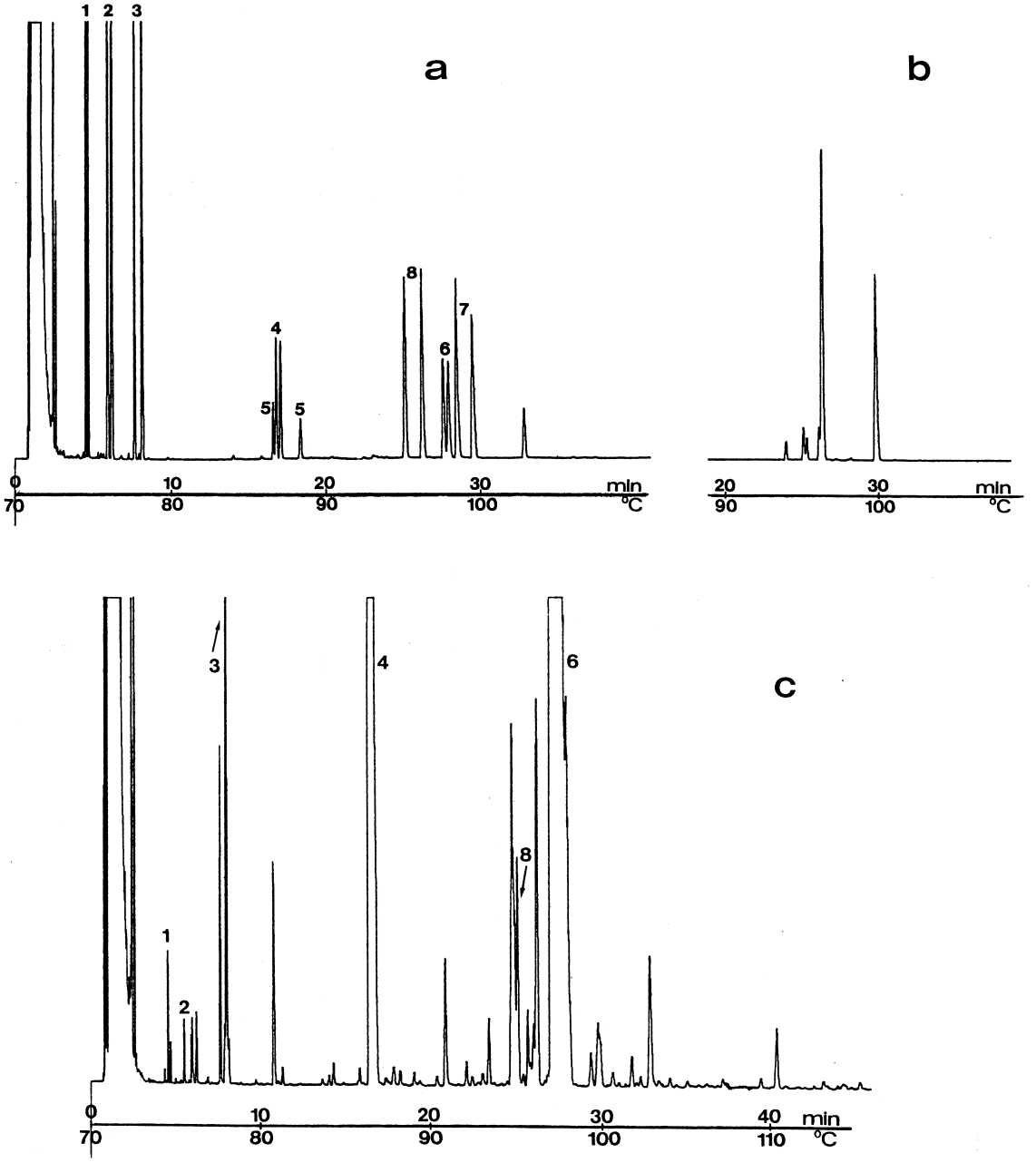

Fig. 2. Es-GC analysis of peppermint EO (column: 30% 2,3-DiMeTBS-β-CD/PS-086): (a) GC pattern of the standard racemates of α-pinene (1), β-pinene (2), limonene (3), menthone (4), isomenthone (5), menthol (6), isomenthol (7) and pulegone (8); (b) GC pattern of menthyl acetate (9); (c) GC pattern of a commercial sample of peppermint essential oil. From Ref. [7] with permission, ©1997 Wiley.

analytical columns and their loading capacity, scaling-up to the corresponding micropreparative columns and determining the loading capacity for several racemates on a 3 μm, 30% 2,6-DiMe-3-Pe-β-CD/OV-1701 column and a 2 μm, 30% 2,3-DiAc-TBS-β-CD/PS-086 column. They applied Prep-cGC to the isolation of methyl 3-hydroxyhexanoate, methyl-2-methylbutanoate, δ-hexalactone, δ-octalac-

tone, and y-decalactone (the latter from a nature-identical mango aroma), obtaining average yields of about 30% when amounts above 20 μg/μl were injected, and an enantiomeric purity above 90%. [11,12].

Venkatachalam and Cole [13] proposed the use of solid-phase microextraction (SPME) in combination with Es-GC, with CD as chiral selector, for the chromatographic detection of food adulteration, synthetic blends, and quality evaluations of natural flavours and EOs (number and position of substituents were not specified in the text).

Betts [14] evaluated the performance of some Es-GC phases with 26 different EO constituents and found that the performance of columns coated with 3-TFA-, 3-propionyl- and 3-Bu-2,6-DiPe-γ-CDs were comparable, but differed from those of 3-TFA-2,6-DiPe-α-CD and Chirasil-Val.

Casabianca et al. [15] determined the enantiomeric distributions of linalool and linalyl acetate in several plant materials of interest for the flavour industry by Es-GC with both 2,6-DiMe-3-Pe-β-CD and 2,3-DiEtTBS-β-CD. They investigated different plant cultivars submitted to four extraction processes: steam distillation, solvent extraction, supercritical fluid extraction, and headspace analysis. Their results showed that careful attention must be paid to linalool, which undergoes partial racemisation under certain processing conditions. Dugo et al. [16] used Es-GC with 2,3-DiEtTBS-β-CD to determine the enantiomeric distribution of linalool to evaluate the authenticity and genuineness of bergamot oil.

Through GC, Es-GC and GC–MS, Manzardo et al. [17] found all four stereoisomers of (3a–7a)-*cis*-3-butylhexahydrophthalide in diethyl ether extracts from celeriac (*Apium graveolens* L. var. *rapaceum*), and assigned the relative configuration of the diastereomers by NMR. TBS-β-CD was used as chiral selector.

In a study dealing with the use of dill and annual and biennial caraway as carvone suppliers for sprouting inhibition in potatoes, Bouwmeester et al. [18] determined the enantiomeric compositions of limonene, carvone, and carveols, in hydrodistillates and hexane extracts of varieties and accessions of seeds of the three vegetable matrices harvested over several years. Both enantiomers of the investigated compounds were detected in the two caraway types and, except for (−)-*cis*-carveol, also in dill. Es-GC was by 2,3-DiPe-6-Me-γ-CD.

Hiltunen and Laakso [19] investigated the enantiomeric composition of monoterpene hydrocarbons of the needle oils of Scots pine (*Pinus sylvestris* L.) and juniper (*Juniperus communis* L.) grown in Finland. Es-GC was by 2,6-DiPe-3-Bu-γ-CD. In both species, the average proportions of (+)-enantiomers was above 50%. The (−)/(+)-ratio of α-pinene (0.4) was the same in low and high Δ-3-carene Scots pine chemotypes; Δ-3-carene occurred entirely as (+)-enantiomer. Multivariate analysis was used to elaborate (+)- and (−)-enantiomeric series, and grouped (−)-α-pinene, tricyclene and (−)-camphene for the (−)-series, and (+)-α-pinene and (+)-β-pinene for the (+)-series, in agreement with the well known biogenetic pathway.

Hiltunen et al. [20] analysed the *n*-hexane extracts of two root samples and 33 seed samples of *Angelica archangelica* L. collected from different parts of Finnish Lapland, using GC–MS and 2,3-DiPe-6-Me-β-CD as chiral selector. They found (−)-α-pinene (19–42%) and (+)-sabinene (21–28%) as major compounds in the root oils and (−)-β-phellandrene (>60%) together with (+)-sabinene, (+)-α-pinene, myrcene, (−)-α-phellandrene, (−)-α-pinene and (−)-limonene as main components in the seed oils.

In a study aiming to obtain data necessary for more exact geochemical interpretations, and if possible to obtain pertinent paleobiological information in fossilised resin (amber), Armstrong et al. [21] investigated the enantiomeric distribution of the most prevalent chiral monoterpenoid in conifers, i.e. α-pinene, β-pinene, camphene and limonene and in amber, i.e. borneol, isoborneol, and camphene. They determined the *ee* of optically active monoterpenes by Es-GC with a β-CD as chiral selector (substituents were not specified in the text).

Grant et al. [22] investigated the chemical composition of monoterpenes emitted by twigs of Eastern white pine, *Pinus strobus* L., by D-HS–GC and D-HS–GC–MS, since volatile emissions from twigs stimulated oviposition by *Dioryctria abietivorella* (Grote) in laboratory bioassays. Four of the six most abundant monoterpenes elicited a significant oviposition response, in particular myrcene and car-3-ene [mainly the (+)-form], were the most active compounds in both electroantennogram (EAG) and

oviposition bioassays, while (−)-limonene elicited a significant oviposition response but was the least stimulating monoterpene in EAG tests. They determined the *ee* of optically active monoterpenes by Es-GC with TriMe-β-CD as chiral selector.

Ravid et al. [23] determined the enantiomeric distribution of borneol isolated by Prep-pGC from several EOs using TriMe-β-CD as chiral selector. They found high enantiomeric purities of (−)-borneol in *Coridothymus capitatus, Artemisia herba alba, Origanum vulgare, Ocimum canum* and *Tanacetum parthenium* (feverfew) EOs and of (+)-borneol in lavandin essence and in lavandin oil. In the EOs of rosemary cultivars and *Salvia officinalis* they did not find a characteristic distribution of the enantiomers of borneol or its biosynthetic oxidation product, camphor. With the same chromatographic systems, they also investigated the enantiomeric composition of linalool in the EOs of seven *Ocimum basilicum* L. chemotypes, *O. sanctum* L., *O. gratissimum* L., and *O. canum* Sims. of Thai origin, and in commercial basil oils. Enantiomerically pure (*R*)-(−)-linalool was found in cultivars of *O. basilicum* EOs of various origins, and in commercial basil oils, while (*S*)-(+)-linalool was the main enantiomer in *O. canum* and *O. sanctum* EOs [24]. They detected for the first time natural enantiomerically pure (4*R*)-(+)-α-terpineol in the EO of *Micromeria fruticosa* (L.) Druce, and analysed 40 EOs and found among others medium to high enantiomeric purities of the (+)-enantiomer in *Origanum vulgare* (88%) and *O. syriacum* (84%), *Mentha citrata* (69–81%), four *Salvia* species (60–74%), and lavender EOs (62–72%), and relatively high enantiomeric purities of (4*S*)-(−)-α-terpineol in the oils of cinnamon and *Laurus nobilis* L. (80%), *Mentha longifolia* (69%), geranium and Cembra pine (64%) and lemon (67%) [25]. The same authors investigated the enantiomeric distribution of verbenone in several rosemary EOs where it is present in variable amounts (2.3–28.9%), using 2,6-DiPe-3-Bu-γ-CD and TriMe-β-CD as chiral selectors. They found (+)-verbenone either enantiomerically pure or at a very high *ee* [26].

Borg-Karlson et al. [27] collected the floral fragrance of the shrub *Daphne mezereum* in central Sweden by means of HS-technique and GC–MS and Es-GC×GC (TriMe-β-CD). They found (*S*)-(+)-linalool with an enantiomeric purity exceeding 99% as the main constituent (95%) and (2*S*, 5*S*)- and (2*R*, 5*S*)-furanoid and (3*R*, 6*S*)- and (3*S*, 6*S*)-pyranoid linalool oxide isomers in amounts varying between 2–5%. They found also that a fragrance sample of *D. mezereum* and one of (*S*)-(+)-linalool attracted males of the vernal solitary bee species *Colletes cunicularius* and *Andrena cinerea*, while a racemic mixture of the furanoid linalool oxides was only weakly attractive. With the same chiral selector, Demyttenaere and Willemen [28] evaluated the results of biotransformation of (+/−)-linalool to *cis*- and *trans*-furanoid linalool oxide (yield 15–24%) and *cis*- and *trans*-pyranoid linalool oxide (yield 5–9%) with *Aspergillus niger,* in particular *A. niger* ATCC 9142, and found that (*R*)-(−)-linalool yielded almost pure *trans*-furanoid and *trans*-pyranoid linalool oxide (*ee*>95%). Mosandl [29] determined the correct elution order of the furanoid linalool oxides on differently modified CD derivatives (TriMe-β-CD, TriEt-β-CD, 2,3-DiMeTBS-β-CD, 2,3-DiAcTBS-β-CD), in consideration of the important role of these compounds in the characteristic odours of many EOs, in particular lavender oils (*Lavandula angustifolia* Mill.), lavandin oils (*Lavandula intermedia* Emeric ex Loisel) and clary sage EOs (*Salvia sclarea* L.).

Kajiwara et al. [30] determined the absolute configuration of 3-butyl-4-vinylcyclopentene in EOs from the marine brown algae *Dictyopteris prolifera* and *Dictyopteris* spp., by Es-GC with TriMe-β-CD as chiral selector, after synthesising (−)-(3*R*,4*R*)-3-butyl-4-vinylcyclopentene. They found that the natural EO component was the (+)-(3*S*,4*S*)-isomer, with almost 100% *ee*.

In an in-depth study on sesquiterpene cyclase activity in bacteria-inoculated or elicitor-treated cotton cotyledons, Davis and Essenberg [31] found an accumulation of (+)-δ-cadinene, suggesting that (+)-δ-cadinene is an early enzymatic intermediate in the biosynthesis of the sesquiterpenoid phytoalexins by upland cotton. They detected (+)-δ-cadinene by Es-GC–MS using TriMe-β-CD as chiral selector. By Es-GC with 2,3-DiPe-3-TFA-γ-CD, Nakamura et al. [32] found that callus of *Marchantia polymorpha* and *Glycine max* diastereo- and enantio-selectively reduced ethyl 2-methyl-3-oxobutanoate to the corresponding *anti*- and *syn*-(*S*)-hydroxyester, respectively.

Coleman et al. [33] determined menthol authenticity in a wide variety of menthol-containing products, including mouthwash, toothpaste, after-shave lotion, creme de menthe and cigarettes, by Es-GC–SIM-MS. (CD substituents and their position were not specified in the text). The method is based on the fact that menthol occurs naturally in oils of the *Mentha* species in the (1*R*, 3*R*, 4*S*)-(−) form (*l*-menthol), whereas synthetic menthol is available either in the same form or as a racemic mixture (*d*- and *l*-menthol). With this method, as little as 0.01% *d*-menthol in the total menthol concentration can be detected, with a relative standard deviation of about 7%.

2.2. Extracts and flavour and aroma compounds

In a study aiming to identify the most odour-active volatiles in fresh, hand-extracted juice of Valencia late oranges by aroma extract dilution analysis (AEDA), Hinterholzer and Schieberle [34] determined the enantiomeric ratios of α-pinene (TriMe-β-CD), limonene and linalool (2,6-DiMe-3-Pe-β-CD) and ethyl 2-methylbutanoate (2,3-DiMeTBS-β-CD). Within this study, they applied static headspace/olfactometry to the juice under investigation and found (*R*)-α-pinene, (*R*)-limonene, ethyl butanoate, (*S*)-ethyl 2-methylbutanoate and acetaldehyde to be the most odour-active compounds in the headspace above the juice.

Morales et al. [35] found that (*S*)-heptanol (*ee* 100%) was the major component of the pentane/dichloromethane extract of Andes berry (*Rubus glaucus* Benth), a native plant of Central-South America whose berries are used for making jams, juices, and for the flavouring of ice-cream, and yoghurt. Heptanol Es-GC separation was by 2,3-DiAcTBS-β-CD.

Mosandl et al. [36] separated the 1-*p*-menthene-8-thiol enantiomers by Es-GC with a 2,3-DiMeTBS-β-CD in PS-268 as stationary phase and evaluated their sensory properties by Es-GC-olfactometry. They found that (*R*)-1-*p*-menthene-8-thiol influenced the natural odour of grapefruit juice while the (*S*)-enantiomer odour was weak and non-specific.

Engel et al. [37] synthesised γ-thiolactones [5-alkyldihydro-2(3*H*)-thiophenones, alkyl: methyl, propyl, pentyl and heptyl] and δ-thiolactones (6-alkyltetrahydro-2*H*-thiopyran-2-ones, alkyl: methyl, propyl, pentyl and heptyl) to investigate their sensory properties. They achieved enantiomer separation by Es-GC using 2,3-DiMeTBS-β-CD as chiral selector.

Mosandl et al. [38] applied Es-GC and Es-GC-olfactometry with 2,3-DiAcTBS-β-CD as chiral selector to separate directly and to determine threshold values and odour characteristics of 4-*tert*-butyl-α-methyldihydrocinnamaldehyde enantiomers, a widely used component of flowery compositions, particularly lily of the valley and linden types, for the cosmetic field. They also deduced the absolute configuration of each enantiomer from X-ray of the diastereomeric amides generated by reaction of the corresponding carboxylic acid with (*S*)-2-amino-2-phenyl-ethanol, after separation and isolation by HPLC.

Bartschat and Mosandl [39] synthesised and directly separated 2-phenylpropanol, 2-phenylpropanal and 2-phenylpropanal dimethyl acetal, three compounds used in the perfumery for flower composition, using Es-GC and 2,3-DiAcTBS-β-CD or 2,3-DiAcTBS-γ-CD as chiral selector and evaluated their odour characteristics and threshold values by Es-GC-olfactometry.

In a study concerning the quality assurance of wine, Mosandl et al. [40] used Es-GC with 2,3-DiMeTBS-β-CD as chiral selector for enantiomeric recognition of ethyl lactate in wines, using (*S*)-methyl lactate as an internal standard.

3. Two-dimensional gas chromatography and two-dimensional gas chromateography–mass spectrometry

GC×GC is a very useful technique in which groups of components not separated on the first column can automatically and on-line be transferred to a second column coated with a different stationary phase, for further separation. But, in this field, GC×GC is also a highly effective and sophisticated clean-up procedure, where only those components whose *ee* has to be determined are transferred to the second and analytical column coated with the CD chiral selector, thus avoiding peak overlap and interference with correct *ee* determination. GC×GC eliminates interference from sample matrices, avoids contami-

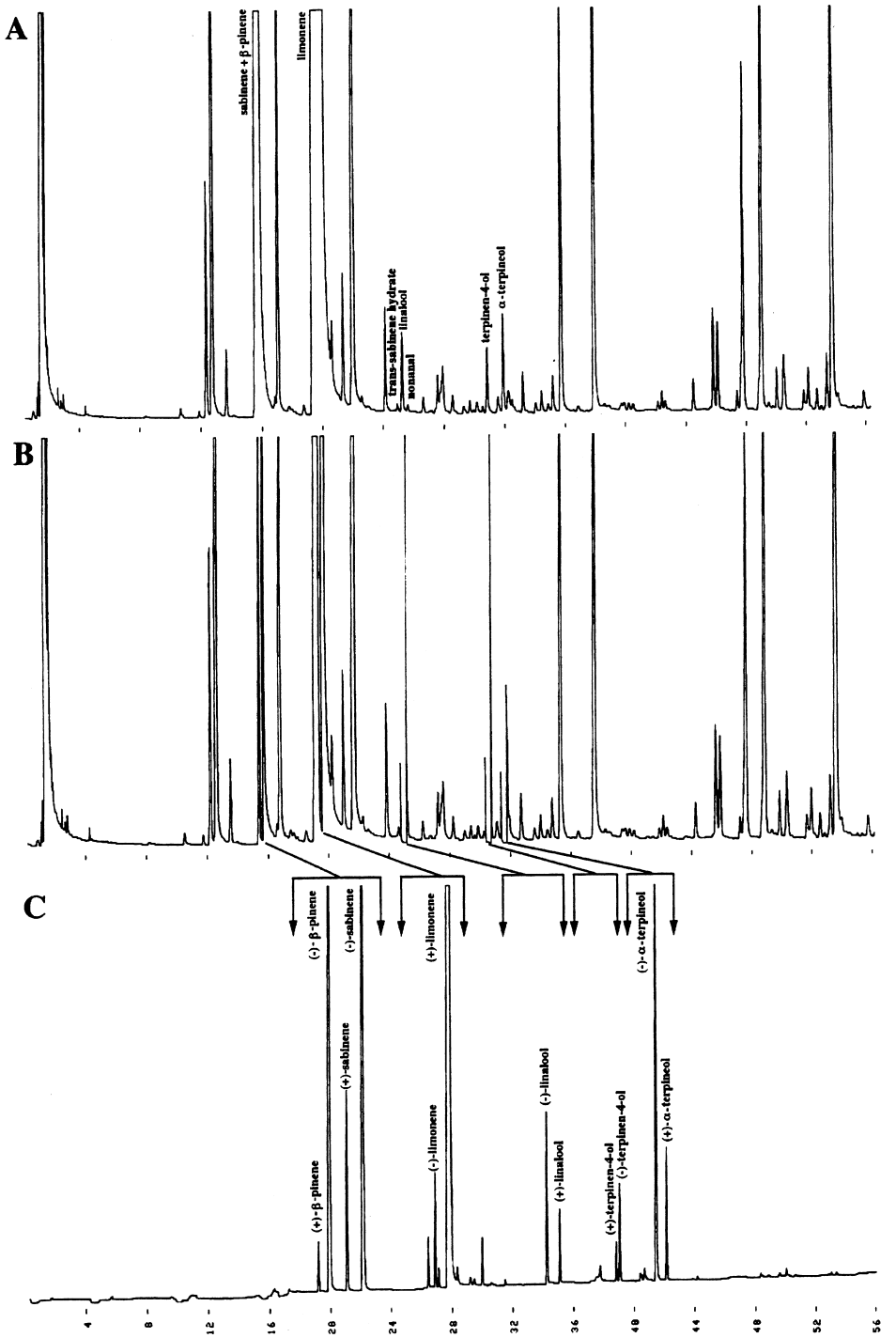

Fig. 3. (A): GC pattern of a cold pressed lime oil (column SE 52); (B): GC pattern of a cold pressed lime oil after five heart-cuts (column SE 52); (C) enantiomer separation of characteristic optically active components using 2,3-DiEtTBS-β-CD as chiral selector. From Ref. [43] with permission, ©1998 Wiley.

nation of the chiral column by high-boiling point components, and provides a simple, rapid, and effective method for stereoanalysis of components from various EOs.

3.1. Essential oils

Wang et al. [41] determined the *ee* of limonene and linalool in various EOs (*Illicium verum*, fennel, rose, myrcia, lemon-grass and orange) by GC×GC using SE-54/TriPe-β-CD and SE-54/2,6-DiPe-3-TFA-α-CD column combinations.

Dugo's and Mosandl's groups studied *Citrus* EOs in-depth through GC×GC. With a fully-automated, double-oven GC×GC system using a SE 52/2,3-DiEtTBS-β-CD/PS-086 column combination, Mondello et al. determined the enantiomeric distribution of β-pinene, sabinene, limonene, linalool, terpinen-4-ol, and α-terpineol in mandarin oils to detect whether extraneous oils or contaminating oils had been added [42], in lime oils (cold-pressed Key lime oils type A and type B, cold-pressed Persian lime oils) distinguishing cold-pressed Key lime oils type A and type B, cold-pressed Persian lime oils, and distilled lime oils [43], and in lemon, mandarin, sweet and bitter orange and bergamot [44]; in addition to the above, in bergamot oil, linalyl acetate was analysed. With the same column combination, P. Dugo et al. [45] evaluated the same components in laboratory extracted oil from three new lemon hybrids and compared the results with those obtained for Italian industrial lemon oils. In a study on the composition of the EO of Uruguayan *Citrus clementine* Hort. (Nules and Comune cultivars), Verzera et al. [46] determined the enantiomeric distribution of β-pinene, sabinene, limonene, linalool and α-terpineol by GC×GC using 2,3-DiEtTBS-β-CD in PS-086 as chiral selector. Fig. 3 shows the GC×GC patterns of a cold pressed lime oil and of the characteristic optically active components [43].

Blanch and Nicholson [47] determined the *ee* of limonene and limonene-1,2-epoxide, which is a trace component in lemon peel samples, by GC×GC and GC–MS using a PS-255/TriMe-γ-CD column combination. They measured an *ee* of (*R*)-(+)-limonene between 97.1 and 97.4% and of (1*S*,2*R*,4*R*)-(+)-limonene-1,2-epoxide between 88.0 and 91.9%.

Mosandl's group investigated in-depth the enantiomeric composition of both 3-butylphthalide enantiomers and all eight 3-butylhexahydrophthalide stereoisomers, which are the character impact flavour compounds of celery and celeriac, which are varieties of *Apium graveolens* [48–50], using Es-GC and GC×GC, and their sensory characteristics and odour thresholds via Es-GC-olfactometry. The absolute configuration of each 3-butylphthalide enantiomer was obtained via ^{1}H-NMR of pure (*R*)-2-phenylpropionic acid diastereomeric derivatives isolated by preparative HPLC. 3-Butylphthalide enantiomers were separated by 2,6-TBS-γ-CD as chiral selector; (3*S*)-enantiomer showed a significantly lower GC odour threshold value than does the (3*R*)-enantiomer, while the (3*S*)/(3*R*) ER in celery seed EO was about 95:5 [48]. They synthesised racemic standards of 3-butylhexahydrophthalide from racemic 3-butylphthalide and separated 3-butylphthalides enantiomers and all eight 3-butylhexahydrophthalide stereoisomers simultaneously by a 2,3-DiAcTBS-β-CD chiral selector, defined their odour characteristics and thresholds by Es-GC-olfactometry and determined their enantiomeric distributions in celery, celeriac, celery seed and fennel extracts [49,50]. Fig. 4 shows the enantiomer separation of butyl(hexahydro)butylphatileds by GC×GC with a PS-268/2,3-DiAc-TBS-β-CD in PS-268 column combination [49].

Sjòdin et al. [51] determined the relative amounts of volatiles, mainly monoterpene hydrocarbons, in eight different tissues of each of four individuals of *Pinus silvestris*. The four trees presented widely different monoterpene compositions. They used GC×GC with TriMe-β-CD or 2,6-DiPe-3-Bu-γ-CD as chiral selectors to determine the enantiomeric composition of α-pinene, camphene, β-pinene, sabinene, β-phellandrene, limonene, and Δ-3-carene, and found large differences in the relative amounts of the monoterpene hydrocarbons as well as in their enantiomeric ratios, both within and between individuals. One-year-old phloem, bark of branches and the shoots of some of the trees showed extremely large relative amounts of (+)-Δ-3-carene or (−)-limonene; in the xylem of the trunk of root or the needles of the same trees only (+)-Δ-3-carene was detected. Using the same analysis techniques and conditions, Borg-Karlson et al. [52] determined the

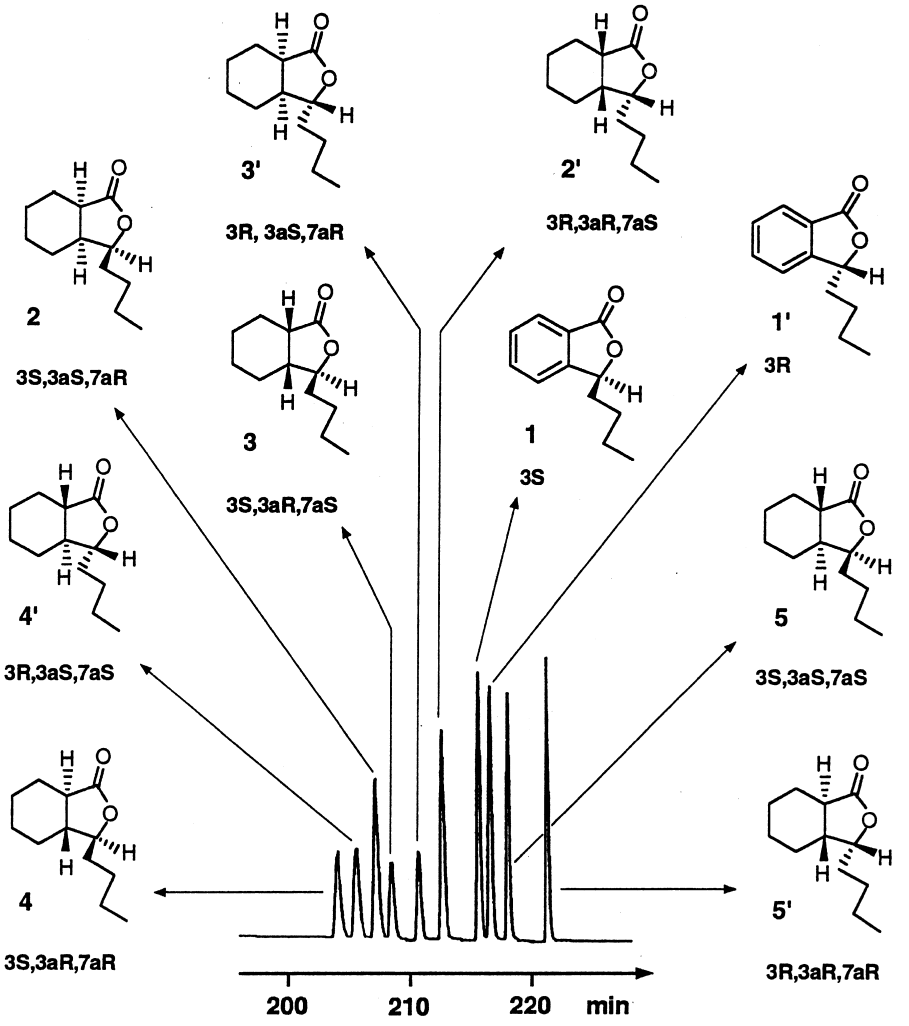

Fig. 4. Enantiomer separation of butyl(hexahydro)butylphatileds by GC×GC with a PS-268/2,3-DiAcTBS-β-CD in PS-268 column combination. From Ref. [49] with permission, ©1997 American Chemical Society.

relative amounts of 23 monoterpene hydrocarbons, and of the previous seven major chiral monoterpenes, present in extracts of branch xylem and needles from 41 *Picea abies* plus-trees of widely different origins. Data were elaborated by multivariate statistical analysis. Enantiomeric compositions and relative amounts of the monoterpenes varied widely both within and among the trees. In the xylem, (−)-β-pinene and (−)-β-phellandrene, while in the needle samples, (−)-α-pinene, (−)-limonene and (−)-camphene, dominated over their (+)-enantiomers.

Schwab et al. [53] investigated the composition of 12 EOs from Madagascar, *Cinnamomum camphora*, *Cinnamomum zeylanicum*, *Hedychium flavum*, *Helichrysum gymnocephalum*, *Helichrysum selaginifolium*, *Lantana camara*, *Pelargonium roseum*, *Piper nigrum*, *Ravensara aromatica*, *Vetiveria zizanioides* and *Zingiber officinale* by GC and GC–MS. In each EO, they detected at least one of five chiral monoterpenes (citronellol, limonene, linalool, terpinen-4-ol and α-terpineol) finding a specific *ee* for each of them by applying Es-GC×GC with 2,3-DiAcTBS-β-CD as chiral selector.

Koenig's group has done fundamental work in the identification and enantiomer recognition of sesquiterpene hydrocarbons in EOs. Recently, Koenig and Joulain [54] have collected the analytical and spectroscopic data of about 330 sesquiterpene hydrocarbons, including the enantiomer recognition of the optically-active ones. Koenig's group studied in-depth the composition of the sesquiterpene hydrocarbon fraction of several species of liverworts (Hepaticae). They obtained racemic sesquiterpenoid standards by synthesis or isolation of the individual enantiomers of opposite configuration from higher plants and liverworts by Prep-pGC and/or classical methods, and analysed them by Es-GC×GC. They used 2,6-DiMe-3-Pe-β-CD to separate the enantiomers of 25 sesquiterpenoids from several plants, in particular *Meum athamanticum*, *Preissia quadrata*, *Lophocolea heterophylla* [55]. The results were used as indicative criteria for chemotaxonomy and to detect adulterations and to obtain new insights in biosynthetic pathways. Koenig et al. [56] first identified β-bazzanene and (−)-α-barbatene and (+)-β-barbatene, a typical constituent of liverworts, as constituents of a higher plant, in the roots of *Meum athamanticum*. In addition, (−)-isobazzanene and (−)-isobarbatene, (−)-β-elemene, (+)-bicyclogermacrene, (−)-germacrene D, (−)-α-chamigrene and (+)-β-bisabolene were also identified. For both GC×GC and Prep-pGC, 2,3-DiMe-3-Pe-β-CD was used. Fig. 5 shows GC and Es-GC×GC analysis of α- and β-barbatene fraction from *M. athamanticum* root and *Bazzania trilobata* EOs [56].

They synthesised pure racemic β-caryophyllene, isolated pure enantiomers by Prep-pGC with 2,6-DiMe-3-Pe-β-CD and analysed its enantiomeric composition in several liverworts [57]. Pure (−)-β-caryophyllene was found in the liverworts *Fossombronia alaskana*, *Trichocolea tomentella* and *Ptilidium pulcherrimum,* as well as in hop, black pepper, clove leaf, copaiva balsam and opoponax EOs, while different enantiomeric excesses of (+)-β-caryophyllene up to 85% were found in *Pellia epiphylla*, and *P.endiviifolia* of different origins and in *Metzgeria conjugata*. With the same chromatographic techniques and using 2,6-DiMe-3-Pe-β-CD and 2,3-DiPe-6-Me-γ-CD as chiral selectors, Koenig et al. [58] investigated the enantiomeric composition of the sesquiterpene hydrocarbon fraction of the liverwort *Lepidozia reptans*. They detected (+)-α-barbatene, (−)-β-barbatene, (−)-bicyclogermacrene, (+)-β-bourbonene (72% *ee*), (−)-cuparene, (−)-α-cuprenene, (+)-β-elemene and racemic δ-elemene and also established the structure and configuration of a new sesquiterpene alcohol, (+)-1(10)-spirovetiven-7β-ol, by spectroscopic methods, chemical conversion and Es-GC. The same group isolated the labile sesquiterpene hydrocarbon (−)-(1*R*,7*S*,10*R*)-cadina-3,5-diene from manuka oil (*Leptospermum scoparium*), and its enantiomer from a chemotype of the liverwort *Conocephalum conicum* by Prep-pGC using 2,6-DiMe-3-Pe-γ-CD and 6-Me-2,3-DiPe-γ-CD as chiral selectors [59]. Structure and absolute configuration were derived by NMR, Es-GC (with 2,6-DiMe-3-Pe-β-CD) and chemical conversion. In addition, in the same EO, they also identified (+)-δ-amorphene, (−)-bicyclosesquiphellandrene, (−)-cadina-1(6),4-diene, (−)-zonarene and (−)-*t*-calamenene. With the same techniques and using 2,6-DiMe-3-Pe-β-CD as chiral selector, they isolated and determined the enantiomeric composition of several sesquiterpene hydrocarbons in the liverwort *Dumortiera hirsuta* (Sw.) Nees, including (−)-cyclosativene, (+)-β-copaene, (−)-aristolochene, (+)-aciphyllene and (−)-isoguaiene, (+)-α-copaene, (+)-*cis*-α-bergamotene, (+)-*trans*-α-bergamotene, (+)-α-guaiene, (+)-guaia-6,9-diene and (+)-β-caryophyllene [60]. They also identified (−)-cuparene, (−)-α-cuprenene and (−)-sesquiphellandrene in the EO of the liverwort *Mannia fragrans* and isolated and identified (−)-α-microbiotene, (+)-β-microbiotene and (−)-cyclocupar-9-en-2-one, correlating them with constituents of opposite configuration from *Microbiota decussata* (Cupressaceae) [61]. Rieck and Koenig [62] elucidated the structure of a new furano-eudesmane alcohol (furano-eudesma-4(15),7,11-trien-5α-ol) from the liverwort *Lophocolea heterophylla* isolated by Prep-pGC with 2,3-DiPe-3-Me-γ-CD. Koenig's group [63] also investigated the sesquiterpene constituents of the liverwort *Preissiaquadrata* using Es-Prep-pGC (2,3-Di-Pe-6-Me-γ-CD) and Es-GC and Es-GC×GC. The main constituent of the chemotype investigated collected in Southern Germany, is the labile germacrene C, which rearranges to racemic δ-elemene with temperature, and (+)-germacrene D, (+)-*trans*-β-caryophyllene, (+)-β-caryophyllene oxide, (+)-

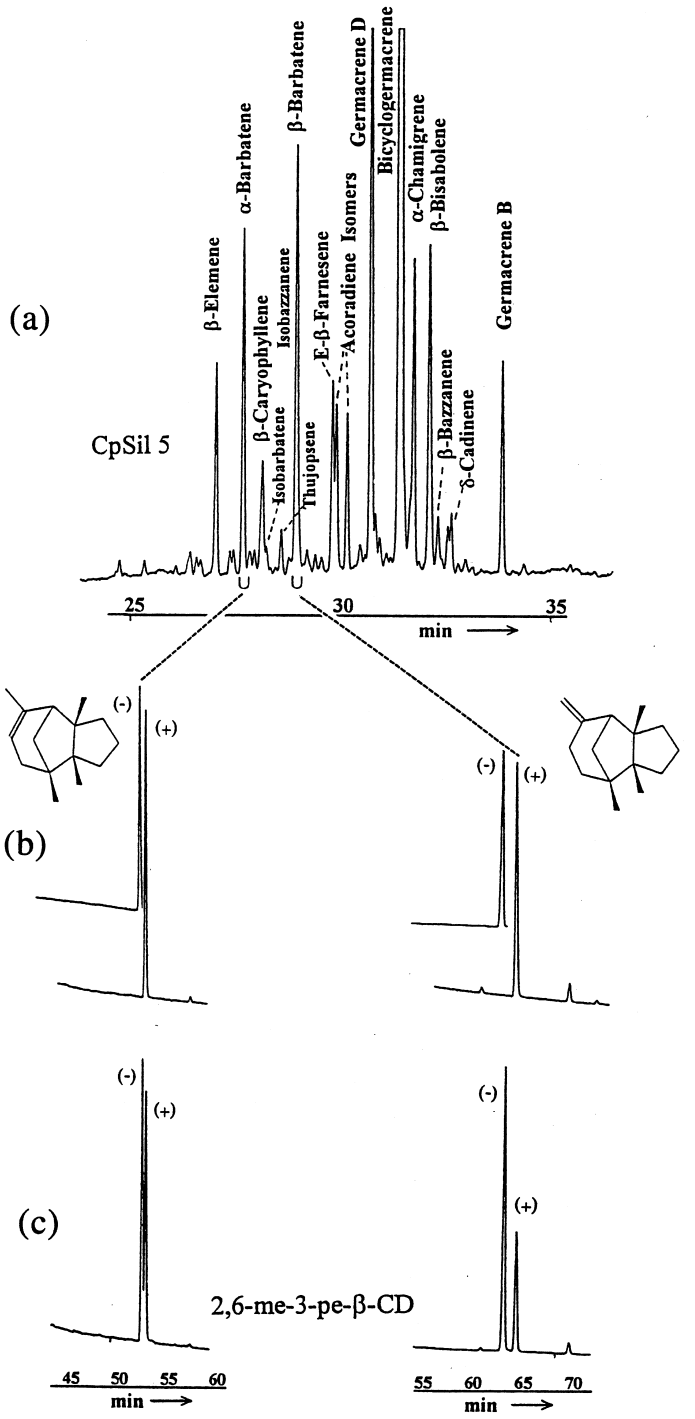

Fig. 5. GC analysis of the EO from *Meum athamanticum* (L.) roots. (A) GC pattern of the sesquiterpene hydrocarbon part (column CpSil 5); (B) Enantiomer separation of α- and β-barbatene fraction from *M. athamanticum* root and *Bazzania trilobata* EOs by GC×GC analysis with a CpSil 5/2,6-DiMe-3-Pe-β-CD; (C) Enantiomer separation of α- and β-barbatene fraction from co-injected *M. athamanticum* root and *B. trilobata* EOs with the same GC×GC system. From Ref. [56] with permission, ©1996 Elsevier.

cubebol and racemic alismol [1β,5α-guaia-6,10(15)-dien-4-ol], and (+)-α-copaene, (+)-δ-selinene, (−)-δ-cadinene, germacrene B and (−)-cascarilladiene (eudesma-5,7-diene) as minor components. With the same chromatographic methods and conditions, Koenig et al. [64] isolated and elucidated the structure of a new eremophilane-type sesquiterpenoid, (−)-1(10),11-eremophiladien-9β-ol, from the liverwort *Marchantia polymorpha* sspp. *aquatica*. They also elucidated the structure of a new epoxy-trinoreudesmane sesquiterpene, (+)-(4*S*,4a*S*,5*R*,8a*S*)-*trans*-4,8a-dimethyl-4a,5-epoxydecalin, from the liverwort *Lophocolea bidentata* collected in Northern Germany and proved its configuration by Es-GC [65]. For both isolation by Prep-pGC and Es-GC they used 2,6-DiMe-3-Pe-β-CD as chiral selector.

From the EO of *Cyperus alopecuroides* from Cameroon, Kubeczka et al. [66] isolated cyprotene, 2,4-patchouladiene, epoxycyperene, 2,4,11-eudesmatriene and 3,5,11-eudesmatriene by Prep-pGC with 2,3-DiPe-6-Me-γ-CD and 2,3-DiMe-6-THS-β-CD and characterised their structures by spectroscopic methods and chemical conversions. Koenig et al. [67] isolated a new eudesmane sesquiterpene (−)-eudesma-1,4(15),11-triene from the EO of cypress pine *Callitris intratropica*. Its conformation was confirmed by Es-GC using 2,3-DiMeTBS-β-CD. In an in-depth study on the *Cedrela odorata* L. EO [68], Koenig's group investigated the enantiomeric composition of several sesquiterpene hydrocarbons, and classified them in four groups. The first group included those with high enantiomeric purity, i.e. (−)-α-cubebene, (−)-α-copaene, (−)-β-caryophyllene, (+)-calarene, (+)-ledene, (+)-bicyclogermacrene, (+)-β-selinene, (−)-α-selinene, (−)-β-bisabolene; the second group those with low enantiomeric purity, i.e. (−)-alloaromadendrene, (+)-γ-muurolene, (+)-α-curcumene, (+)-δ-cadinene, (−)-cadina-1,4-diene, (−)-*trans*-calamenene. The third group contained compounds enriched with the less usual enantiomer, i.e. (+)-*cis*-calamenene, (+)-α-muurolene, and (−)-α-calacorene, while the fourth group comprised achiral or racemic compounds, i.e. δ-elemene, α-humulene, β-farnesene and germacrene B.

Koenig's group [69] also isolated enantiomeric diterpene hydrocarbons from different plants by Prep-pGC and separated them by Es-GC using either 2,6-DiMe-3-Pe-β-CD or 2,3-DiMeTBS-β-CD as chiral stationary phases. In particular they isolated (−)- and (+)-16-kaurene from *Cryptomeria japonica* and *Podocarpus spicatus*, (−)- and (+)-beyerene from *Araucaria araucana* and *Thuja occidentalis*, and (−)- and (+)-7,13-abietadienes from *Pinus pseudostrobus* and *Pellia epiphylla*. Fig. 6 shows Es-GC separation of 16-kaurenes, beyerenes and 7,13-abietadienes.

Mosandl et al. [70] using Es-GC×GC with a PS-268/2,3-DiAcTBS-β-CD column combination separated the four stereoisomers of (*E,Z*)-2,3-dihydrofarnesals and found that the (3*S*)-enantiomer of (*E*)-2,3-dihydrofarnesals is the prevalent enantiomer in the scent of the orchid *Aerides jarchianum* and in the blossom fragrance of *Citrus limon*. They synthesised racemic (*E,Z*)-2,3-dihydrofarnesals, and determined the absolute configuration of each enantiomer by NMR experiments after chemical trans-

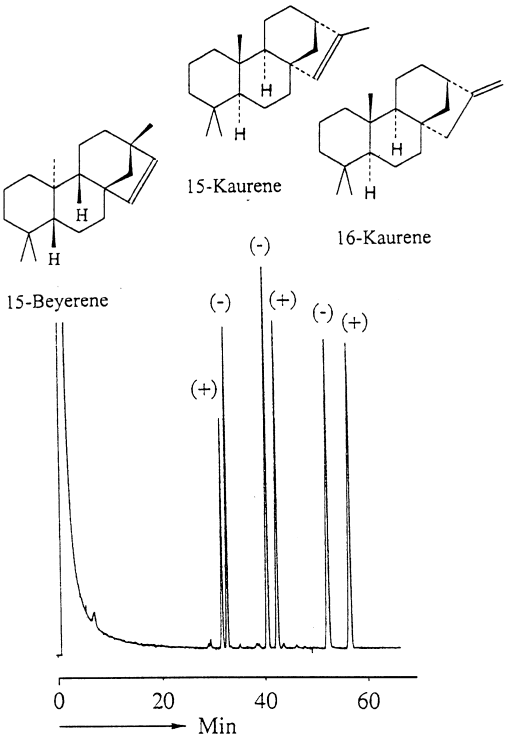

Fig. 6. Es-GC separation of the diterpene hydrocarbons 16-kaurene, beyerene and 7,13-abietadienes using 2,6-DiMe-3-Pe-β-CD as chiral selector. From Ref. [69] with permission, ©1997 Wiley.

formation, diastereomerisation and isolation of pure diastereomers by Prep-HPLC.

Mosandl et al. [71] used Es-GC×GC with a PEG/2,3-DiMeTBS-β-CD or a PEG/2,3-DiAcTBS-β-CD column combinations for the simultaneous stereodifferentiation of important chiral compounds of *Rosmarinus officinalis* L. EO. In particular they investigated α-pinene, camphene, β-pinene, limonene, borneol, terpinen-4-ol, α-terpineol, linalyl acetate, linalool and camphor in authentic and in commercially available rosemary oil samples of different origins, and showed that (l*S*)-(−)-borneol of high enantiomeric purity (>90%) is a reliable indicator of genuine rosemary oils. Mosandl's group [72] enantioselectively synthesised all four stereoisomers of *cis*- and *trans*-rose oxide ketones and investigated their enantiomeric distribution in geranium EOs of different origins by Es-GC using 2,3-DiAcTBS-β-CD as chiral selector. They proposed and demonstrated a pathway for the biogenesis of rose oxide in *Pelargonium graveolens* l'Héritier and *Pelargonium radens* H.E. Moore, by feeding experiments using deuterium labelled citronellyl glucosides and [1,1-$^{2}H_{2}$,^{18}O] citronellol as precursors. The volatile fraction of the plants was analysed using SPME and Es-GC×GC–MS [73,74].

Winterhalter et al. [75] isolated and characterised 20 monoterpenic diols and rose oxides in a subfraction from the methanolic extract of petals of *Rosa damascena* Mill. In addition to the above, they elucidated the structure of (*S*)-3,7-dimethyl-5-octene-1,7-diol and demonstrated that it is the chief precursor of rose oxides. The (*S*)-configuration was assigned from the configuration of the resulting rose oxides using Es-GC×GC with a PEG/2,3-DiMeTBS-β-CD in SE-54 column combination.

Mosandl's group [76] used Es-GC×GC with 2,3-DiAcTBS-γ-CD as chiral selectors for the simultaneous stereo-differentiation of the chiral major compounds of buchu leaf EO [*Barosma betulina* (Bergius) *pillans* or *B. crenulata* (L.) *pillans*]. They directly determined the enantiomeric distributions of limonene, menthone, isomenthone and pulegone, and of the characteristic flavour impact compounds, 3-oxo-p-menthane-8-thiol and its thiol acetate in commercially available EOs as well as laboratory prepared samples and discussed their use as an indicator of authenticity of 'cassis' type fruit aromas and fragrances.

3.2. Extracts and flavour and aroma compounds

Mosandl et al. [77,78] determined the enantiomeric distributions of 3-mercaptohexanol and 3-methylthiohexanol in yellow and purple passion fruits by Es-GC with sulphur-selective detection (SSD) and GC–MS using 2,3-DiAcTBS-β-CD and 2,3-DiMeTBS-β-CD as chiral selectors. They found a variable *ee* of the (*S*)-enantiomer for 3-mercaptohexanol and a high enantiomeric purity of the (*S*)-enantiomer for 3-methylthiohexanol. The same group [78,79] determined the enantiomeric distributions of *cis*- and *trans*-2-methyl-4-propyl-1,3-oxathiane and 3-mercapto-hexyl acetate and butanoate by Es-GC×GC–SSD; 2,3-DiBuTBS-γ-CD was used as chiral selector. They detected 4*S*-configured oxathianes and *S*-configured 3-mercapto-hexyl alkanoates of high enantiomeric purity and quantified them in a range from less than 0.5 to 18 ppb using 2,2-dimethyl-4-propyl-1,3-oxathiane and 3-mercaptohexyl propanoate as internal standard. They therefore proposed using these compounds to differentiate between naturally occurring flavour compounds and synthetic racemates added to passion fruit extracts and products. Fig. 7 shows the structures of 4-propyl-1,3-oxathianes and mercaptohexyl esters in yellow passion fruit and their GC×GC–SSD analysis using 2,3-Bu-TBS-β-CD as chiral selector [79].

Weber and Mosandl [80] synthesised enantiopure 3-methylthioalkanals, in particular, 3-methylthiobutanal enantiomers, and attributed their absolute configuration. They determined the odour characteristics of the enantiomers by Es-GC-olfactometry using 2,3-DiBuTBS-γ-CD as chiral selector and found that only the (*R*)-configured 3-methylthiobutanal exhibits the typical odour of cooked potatoes, whereas the (*S*)-configured stereoisomer is odourless. Mosandl [81] evaluated the 'moving column stream switching' (MCSS) GC×GC system (using 2,3-DiMeTBS-β-CD as chiral selector) by applying it to the ER determination of γ- and δ-lactones in apricot aroma and characterising sulphurated compounds in passion fruit extracts.

Mosandl et al. [82] applied Es-GC×GC (using 2,3-DiMeTBS-β-CD as chiral selector) to the analysis of the scent of the living, white flowering orchid *Aerangia confusa* and found that *cis*-(4*S*)-methyl-(5*S*)-decanolide is the only genuine stereoisomer of Aerangia lactone. They synthesised racemic *cis*- or

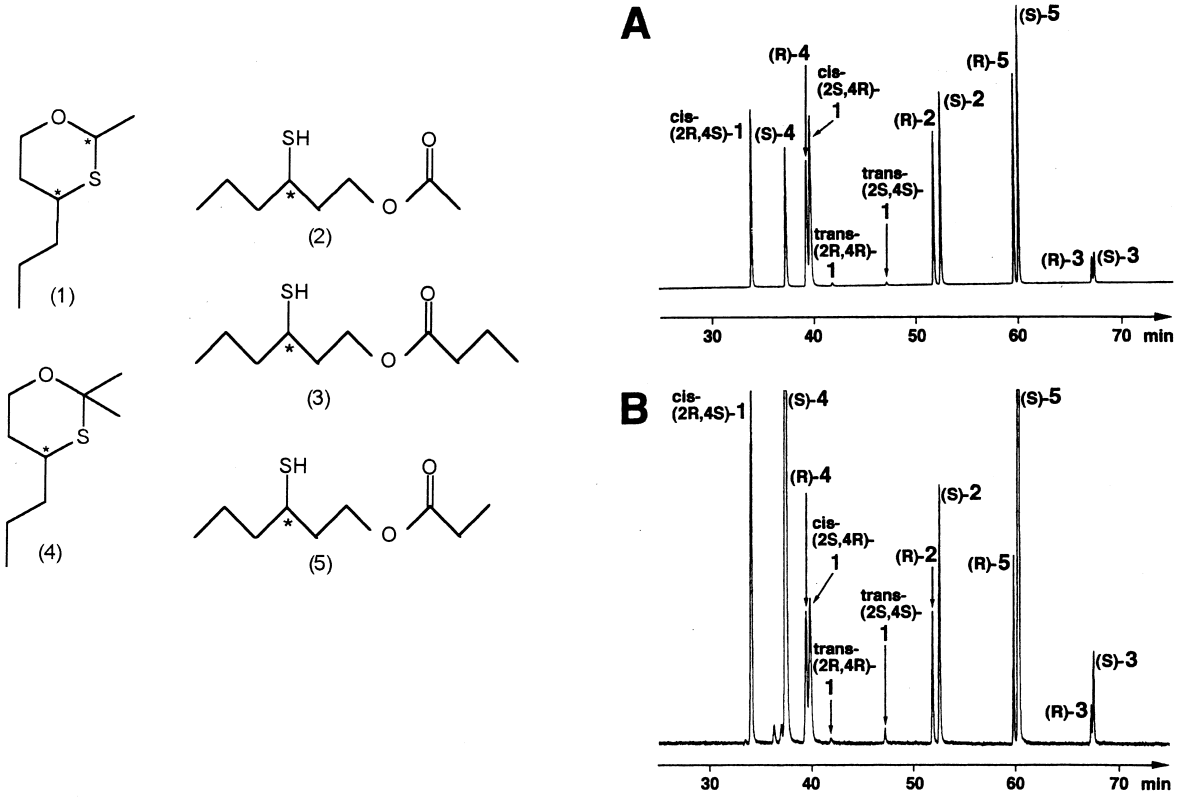

Fig. 7. Structures of 4-propyl-1,3-oxathiane and mercaptohexyl esters in yellow passion fruit; GC×GC–SSD of stereoisomers 1–5 using 2,3-BuTBS-β-CD as chiral selector; (A) standard racemates; (B) mixture of the aroma extract from a passion fruit nectar and the standard solution (co-injection). From Ref. [79] with permission, ©1995 American Chemical Society.

trans-4-methyl-5-decanolide, isolated the pure enantiomers by Prep-HPLC, and determined their absolute configuration by NMR experiments after chemical transformation and diastereomerisation.

Mosandl et al. [83] measured the enantiomeric distribution of δ- and γ-lactones from dairy products, margarine and coconut by Es-GC×GC with 2,3-DiAcTBS-β-CD as chiral selector, reporting for the first time the simultaneous stereodifferentiation of the δ- and γ-lactones from C_{13} to C_{18}.

Mosandl's group described a general approach to the synthesis of 2-, 3- and 4-alkyl-branched acids with high enantiomeric purity and attributed their absolute configuration [84]. They analysed 4-ethyloctanoic and 4-methylheptanoic acid by Es-GC with 2,3-DiMeTBS-β-CD as chiral selector, and determined the sensory properties of several 4-alkyl-branched acids by Es-GC-olfactometry with 2,3-DiMeTBS-γ-CD. They then determined the enantiomeric distribution of various 2-, 3- and 4-alkyl-branched acids in Roman chamomile (*Anthemis nobilis* L.), Parmesan cheese, mutton and read meat flavour, by Es-GC×GC and Es-GC×GC–MS [85].

Schreier et al. [86] investigated the enantiomeric distribution of 2-pentanethiol in guava fruit (*Psidium guajava* L.) by Es-GC×GC with sulphur chemiluminescence detector and 2,3-DiEtTBS-β-CD as chiral selector. (*S*)-2-Pentanethiol was found to be the main component responsible for the characteristic 'tropical' aroma of this fruit.

Schwab et al. [87] evaluated the enantiomeric distribution of free and glycosidically bound octane-1,3-diol and 5(*Z*)-octene-1,3-diol, as well as ethyl 3-hydroxyoctanoate and ethyl-5(*Z*)-3-hydroxy-octenoate in liquid–liquid extracts from five apple cultivars (Renao; Bedan; Peau the Chien; Noel des Champs; Red Delicious) and after enzymatic hydrolysis. Es-GC×GC was by a PEG/2,3-DiAcTBS-

β-CD column combination. They found *ee*'s above 99% of the (*R*)-configuration for the free diols in the cvs. Renao, Bedan, Peau de Chien and Noel des Champs, for the bound diols in the cvs. Bedan, Peau de Chien and Noel des Champs and for the hydroxyesters in cvs. Noel des Champs and Red Delicious by comparison of retention times of synthesised optically-enriched reference compounds with isolated diols and hydroxyesters. Under the same analysis conditions, Beuerle and Schwab [88] identified octane-1,3-diol, 5(*Z*)-octene-1,3-diol, methyl 3-hydroxyoctanoate, ethyl 3-hydroxyoctanoate and ethyl 5(*Z*)-3-hydroxyoctenoate in seven pear (*Pyrus communis* L.) cultivar extracts obtained by liquid–liquid extraction. The 3-hydroxy derivatives all exhibited enantiomeric excesses above 99%. They also detected (2*S*,4*R*)- and (2*R*,4*R*)-2-methyl-4-pentyl-1,3-dioxane, as well as (2*S*,4*R*)- and (2*R*,4*R*)-2-methyl-4-(2′(*Z*)pentenyl-1,3-dioxane, products formed by acetaldehyde and octane-1,3-diol or 5(*Z*)-octene-l,3-diol, respectively, in pear fruits. Schreier et al. [89] identified 1,3-dioxanes (i.e. (2*S*,4*R*)- and (2*R*,4*R*)-2-methyl-4-pentyl-1,3-dioxane, (2*S*,4*R*)- and (2*R*,4*R*)-2-methyl-4-(2′(*Z*)-pentenyl)-1,3-dioxane) in French cider extracts obtained by liquid–liquid extraction by Es-GC and Es-GC–MS. They determined their absolute configuration and conformation by NMR techniques, Es-GC×GC with 2,3-DiAcTBS-β-CD as chiral selector, and by comparison with synthesised reference compounds.

Schreier et al. [90] synthesised the Riesling acetals from their natural precursor, 2,6,10,10-tetramethyl-1-oxaspiro[4,5]-dec-6-ene-2,8-diol, isolated their diastereomeric esters with (−)-α-methoxy-α-trifluoromethylphenylacetic acid by Prep-HPLC, and from each of them obtained their pure enantiomers after chemical transformation and preparative GC using TriMe-β-CD as chiral selector. They also determined their absolute configuration by NMR experiments. They used Es-GC×GC with a PEG/TriMe-β-CD and found that the sensory properties of each enantiomer differed: (+)-Riesling acetal showed a weak woody, fruity, ionone flowery note, whereas the (−)-enantiomer exhibited a camphoraceous, flowery note.

Näf et al. [91] identified the two diastereoisomers of sherry-lactone (5-hydroxy-4-hexanolide) and the corresponding ketone (5-keto-4-hexanolide= solerone) in a solvent extract of dried figs (*Ficus carica* L.) and determined their enantiomeric distributions by Es-GC and Es-GC×GC–MS using 2,6-DiPe-3-Bu-γ-CD as chiral selector. The identity of each compound was confirmed by comparison with standards obtained by enantioselective synthesis. Contemporarily, Schreier et al. [92] identified for the first time 4,5-dihydroxyhexanoic acid-γ-lactone isomers (solerol) in concentrates of dried fig and date (*Phoenix dactylifera* L.) fruit extracts. Es-GC–MS and Es-GC×GC with a PEG/2,6-DiMe-3-Pe-γ-CD column combination analysis gave an (4*R*,5*R*)-isomer *ee* of 80 and 90%, in figs and dates, respectively. With the same GC techniques and 2,6-DiMe-3-Pe-β-CD as chiral selector, Schreier's group [93] also determined the enantiomeric distribution in sherry wines of solerone (5-oxo-4-hexanolide), ethyl 4-hydroxy-5-oxohexanoate, ethyl 5-hydroxy-4-oxohexanoate, 4-oxo-5-hexanolide, and solerol (5-hydroxy-4-hexanolide) and discussed the stereochemical results regarding their biogenesis in sherry. They also found that while Es-GC–MS prevented any racemisation of α-ketols caused by keto–enol tautomerisation during analysis, Es-GC×GC (coupled either by live T switching or by MCSS) led to rearranged constitutional- and stereoisomers.

In a study on glycosidically-bound aroma precursors, Schreier et al. [94] isolated ethyl 3-*O*-β-glucopyranosilbutanoate, butyl 3-*O*-β-D-glucopyranosilbutanoate and 3-oxo-octyl-l-*O*-β-D-glucopyranoside from papaya fruit pulp (*Carica pubescens*) and identified them after peracetylation by comparison of GC and GC–MS data with those of synthesised reference compounds. They achieved chiral evaluation of glycosidically-bound-3-hydroxybutanoates and octane-1,3-diol by Es-GC×GC (PEG/2,6-DiMe-3-Pe-β-CD and PEG/2,3-DiAc-TBS-β-CD, respectively) by comparison of retention times of synthesised, optically-enriched reference compounds with enzymically-released aglycones, and found *ee*'s of the (*S*)-3-hydroxybutanoates of 96 and 24% for the ethyl and butyl esters, respectively, and of 90% for (*R*)-octane-1,3-diol.

Schreier et al. [95] synthesised edulans I and II diastereomers, the important flavour components of purple passion fruit (*Passiflora edulis* Sims); they then isolated the optically pure form by analytical HPLC and established their absolute configuration by

NMR. Using Es-GC×GC–SIM-MS (PEG/2,6-Di-Me-3-Pe-β-CD column combination), they determined the enantiomeric distribution of edulans I and II in a number of purple passion fruit extracts and distillates of different origin (Kenya, Chile and Ivory Coasts) and found that the (2*S*)-enantiomers predominated. They also synthesised 3,4-dihydro-3-oxo-edulans, a well known aroma compound of Burley, Turkish and Lanka tobacco and of purple passion fruit, *Lycium chinense* M., oak wood and chardonnay grape juice [96]. Using Es-GC×GC–SIM-MS (PEG/2,6-DiMe-3-Pe-γ-CD column combination), they evaluated the enantiomeric distribution of 3,4-dihydro-3-oxo-edulans in purple passion fruit, in Riesling wine and grapevine leaves, and quince fruit extracts. Neugebauer and Schreier [97] identified a number of C_{13} norisoprenoids generated with acid-catalysed degradation by SDE at pH 2.5 of glycosidically bound 3-hydroxy-α-ionol from stinging nettle (*Urtica dioica* L.) by GC–MS and GC–Fourier transform infrared spectrometry and enantiodifferentiated them by Es-GC×GC–SIM-MS (PEG/2,6-Di-Me-3-Pe-β-CD column combination). Fig. 8 reports the enantiomer separation by GC×GC–MS of two of the C_{13} norisoprenoids under investigation.

Engewald et al. extensively studied α-campholene and fencholene derivatives because of their interesting odour properties, e.g. sandalwood and woody notes. They first analysed 28 derivatives (alcohols, ethers, esters, etc.) with one stereogenic centre [98] and then eight derivatives with two or more stereogenic centres [99] by Es-GC and Es-GC×GC using TriMe-α-CD and TriMe-β-CD as chiral selectors.

4. Gas chromatography– and two-dimensional gas chromatography–isotope ratio mass spectrometry

The combination of the results from GC–IRMS, or better from GC×GC–IRMS, with Es-GC has been shown to be very effective in the authenticity control of natural flavour and fragrance compounds in flavours and EOs, provided that suitable methods and comprehensive data from authentic sources are available. This is because identical δ-C^{13} ratios are expected for enantiomers from genuine compounds, even if the chiral molecules to be analysed are partially racemised, it being improbable that racemic compounds would be synthesised through different biochemical pathways in the same organism. The literature on these topics has been reviewed by Mosandl [100].

4.1. Essential oils

Casabianca and Graff [101] investigated four typical raspberry aroma compounds, α- and β-ionones, δ-decalactone and raspberry ketone, through Es-GC, GC×GC, and GC–IRMS, using 2,6-DiMe-3-TFA-γ-CD as chiral selector. They analysed different raspberry cultivars and commercial raspberry flavoured products, and elaborated the results statistically by principal component analysis. Through these methods, they were able to discriminate between cultivars and natural and adulterated samples.

Mosandl et al. [102,103] reconstructed characteristic authenticity profiles of neroli, petitgrain, bergamot and other citrus oils through Es-GC×GC as well as GC–IRMS data. Linalool, linalyl acetate, α-terpineol (using 2,3-DiAcTBS-β-CD as chiral selector) and α-pinene, β-pinene, limonene, terpinen-4-ol and nerolidol (using 2,3-DiMeTBS-β-CD as chiral selector) were simultaneously stereoanalysed. Characteristic authenticity profiles of neroli and petitgrain oils were obtained [103]. The same authors [104] used Es-GC and GC–IRMS as well as quantitative results, as alternative methods to establish the authenticity of bergamot oil through the ER of α- and β-pinene, limonene, linalool, linalyl acetate and isotopic data of the chiral compounds just mentioned, and of myrcene, γ-terpinene, neryl acetate and caryophyllene. They also discussed the scope and limitations of the techniques adopted. The same group [105] established a characteristic authenticity profile for the authenticity control of commercially available mandarin oils by combining these techniques. They investigated cold pressed mandarin EOs, distilled mandarin and sweet orange oils to assess blends of cold-pressed mandarin oils with these products.

Mosandl's group investigated, in-depth, the EO of dill *(Anethum graveolens* L.). They first developed a new stereoselective synthesis, then the simultaneous enantioselective analysis of dill-ether and its *cis*-

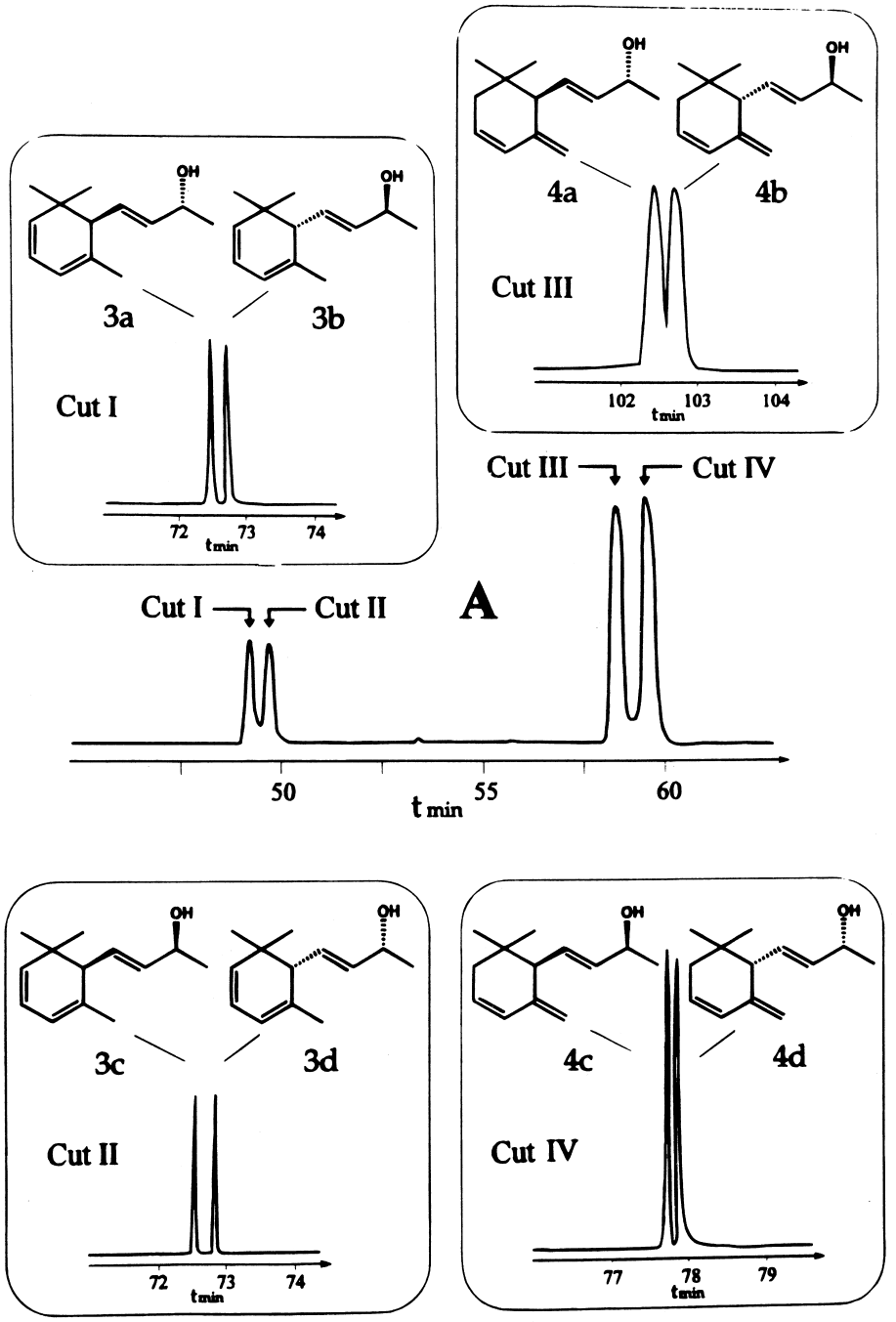

Fig. 8. Enantiomer separation by GC×GC–MS of two of the C_{13} norisoprenoids generated with acid-catalysed degradation by SDE at pH 2.5 of glycosidically bound 3-hydroxy-α-ionol from stinging nettle (*Urtica dioica* L.) (PEG/2,6-DiMe-3-Pe-γ-CD in OV-1701). From Ref. [97] with permission, ©1995 American Chemical Society.

isomers with 2,3-DiBuTBS-γ-CD as chiral selector and determined their olfactory properties [106]. The same group combined $\delta^{13}C_{PDB}$ values and enantiomeric distribution of significant components with EO composition of *Anethum graveolens* at various development stages of different plant parts. They found that the EO composition varies for the various plant parts and changes significantly during the ripening of the dill umbels. Through Es-GC×GC with a OV-215/2,3-DiMeTBS-β-CD column combination, they found enantiomerically pure α-phellandrene, β-phellandrene, dill-ether and carvone, while the enantiomeric composition of limonene varied in all investigated plant parts and changed during development of the umbels. The $\delta^{13}C_{PDB}$ values of the different monoterpenes also change with development. On the basis of these results, they postulated a biochemical pathway of the monoterpenes in dill, and drew some considerations about the biosynthesis of the monoterpenes in the entire plant [107]. Mosandl et al. [108] analysed the EO of ten authentic coriander samples (*Coriandrum sativum* L.) of different origins by GC–IRMS and Es-GC×GC using 2,3-DiMeTBS-β-CD in PS-268 as chiral stationary phase. They compared the ER and the $\delta^{13}C_{PDB}$ values of 12 characteristic authentic EO components with those of commercially available spices and EO to evaluate their authenticity. Mosandl et al. [109] combined Es-GC×GC and GC–IRMS to evaluate origin and achieve authenticity control of balm oil (*Melissa officinalis* L.) components. They used 2,3-DiAcTBS-β-CD as chiral selector to determine the ER of *cis*- and *trans*-rose oxides, citronellal, citronellol and methyl citronellate. The same group [110] used Es-GC–IRMS and Es-GC×GC–IRMS to determine the $\delta^{13}C_{PDB}$ values of some typical chiral and achiral peppermint oil constituents (menthone, isomenthone, menthol, menthyl acetate and 1,8-cineole) and established a characteristic isotopic fingerprint of authentic peppermint oil to be used for authenticity control of commercially available samples. 2,3-DiMeTBS-β-CD in PS-268 was used as stationary phase.

4.2. Extracts and flavour and aroma compounds

Mosandl et al. [111] determined the odour characteristics of chiral 3[*2H*]-furanones by Es-GC and Es-GC-olfactometry using 2,3-DiMeTBS-β-CD/PS-268 as chiral stationary phase and found great differences in the odour quality and thresholds of the single enantiomers. They also determined the origin of 2,5-dimethyl-4-hydroxy-3 [*2H*]-furanone and 2,5-dimethyl-4-methoxy-3[*2H*]-furanone from strawberries, pineapples and commercial samples by GC–IRMS.

5. High-performance liquid chromatography–capillary gas chromatography

On-line LC–GC is a very effective combined technique for the on-line isolation and analysis of specific fractions in complex mixtures. We have only found three applications of LC–GC and all to citrus oils due to Dugo's group.

Dugo et al. [112,113] determined enantiomeric ratios of linalool and terpinen-4-ol in Italian sweet orange oils, Italian and foreign bitter orange oils, Uruguayan mandarin oils, mixtures of sweet and bitter orange oils and lemon oils with a fully automatic on-line LC–GC system. The HPLC preseparation was by a 25 cm×2 mm I.D. Spherisorb 5 μm HPLC column using pentane-*tert*-butyl methyl ether as mobile phase in step elution; Es-GC analysis was by a 2,6-DiMe-3Pe-β-CD column. They also distinguished bitter from sweet orange oils, European (Spain and ltaly) from Brazilian and Ivory Coast bitter orange oils, and cold pressed from distilled lemon and mandarin oils, and obtained information about the cultivars of mandarin and sweet orange oils. In an overview of LC–GC in the analysis of citrus oils, Mondello et al. [114] determined the enantiomeric distribution of linalool and terpinen-4-ol with 2,3-DiMe-6-Pe-β-CD as chiral selector in several lemon, mandarin, bitter orange, sweet orange and mixtures of bitter and sweet orange oils to evaluate their genuineness, quality and origin.

6. Some critical considerations

From the literature which has been considered above, it is clear how important Es-GC with CDs as chiral selectors is to define biosynthetic pathways and chemotaxonomical correlations and classification, to establish origin or genuineness of EOs or

aroma samples and to relate absolute configurations with sensory properties determined through Es-GC-sniffing. In the case of EO or aroma components with naturally variable composition, more accurate results can only be obtained by combining Es-GC results with other data. For a correct determination of genuineness and/or origin, an EO or an aroma can be characterised by simultaneously evaluating the ER of a group of optically active components in the sample under investigation with a fixed enantiomeric composition, that may have been formed through different biogenetic pathways; thanks to the high enantioselectivity of the most recent CD derivatives, the representative optically active components in an EO or an extract can very often be analysed in a single Es-GC run. When this approach is not adequate, or when an even more reliable sample authenticity confirmation is required, ER should be combined with the δC^{13} ratios, which is a more constant parameter, as it is improbable that the different enantiomers of a racemate are biosynthesised through different pathways.

Direct ER determination may sometimes be difficult, since EOs are generally complex mixtures, and Es-GC analysis may double the number of peaks of the optically-active components, thus making the resulting chromatograms even more complex: this increases the probability of peak overlap and interferes with a correct ER determination. ER can correctly be measured by isolating the components under investigation either off-line, through time-consuming chemical procedures, or by preparative Es-GC (or Prep-HPLC) followed by Es-GC, or on-line, by GC×GC or Es-GC–SIM-MS. The most frequently applied technique is GC×GC since, with an appropriate heart-cut, only the components of interest are transferred from the first to the second chiral column. Alternatively, GC–SIM-MS can also be adopted, but suitable specific diagnostic ions must be selected to overcome the interferences due to peaks coeluting with the two (or more) enantiomers: for some reasons, this approach is seldom adopted.

Unfortunately, a universal CD derivative for GC enantioseparation has not yet been found, but a set of two, or better three, columns coated with the most recent CD derivatives makes it possible to separate more than 85% of the racemates in the EO or aroma fields. The latest CDs are not only characterised by a higher enantioselectivity but also by very good GC properties, so that columns are highly stable and their performance reliable over time. In the authors' experience, a symmetrically or asymmetrically alkylated-CD column (e.g. 2,6-DiMe-3-Pe-β-CD) and one or, better, two columns coated with CDs asymmetrically substituted in position 6 with the groups *tert*-butyldimethylsilyl and in positions 2 and 3 with methyl, ethyl or acetyl groups, can satisfy the everyday analytical needs of a laboratory in this field. The set of columns can effectively be chosen with the help of one of the commercially available data bases on Es-GC separation that contains data on most of the separated racemates in this field [115].

On-going research in this field mainly deals with new applications of Es-GC for the identification of which enantiomer of the optically active components is present in an EO or in a plant extract, and in which amounts. Pure enantiomers for spectroscopic investigation are generally obtained from EOs or extracts of plants in which they have been found by direct chemical isolation and/or through preparative Es-GC and/or by stereoselective synthesis, or normal synthesis followed by enantiomer isolation by chemical procedures or by preparative Es-GC or Prep-HPLC. Very interesting work has been done in the field by the groups of Koenig, Mosandl and Schreier.

Several topics are still open but in the authors' opinion, one of the most important is the systematic study of Es-GC of oxygenated sesquiterpenoids and volatile diterpenoids, just as Koenig and Joulain have done for sesquiterpene hydrocarbons [54]. This is a difficult task not only because their number is much higher than that of the corresponding hydrocarbons, but also because most of the oxygenated sesquiterpenoids are medium-to-low volatility racemates, and are thus in the more general and still open problem of high temperatures Es-GC with CDs as chiral selectors. GC enantiomeric separation with CDs is strongly affected by the temperature, and low elution temperatures help to discriminate between the energies of the complexes formed by each of the enantiomers with the CD selector. Separations of medium-to-low volatility racemates may therefore be unsuccessful, not only because the enantioselectivity of the CD used is too low, but also because the elution temperature is too high to discriminate

between the enantiomer-CD complexes. Several authors are dealing with these problems using different approaches including the evaluation of already described CDs at high temperature or the development of new CDs with high enantioselectivity at high temperatures, or by modifying the operative GC conditions or adopting dedicated columns with length, film thickness and inner diameter able to decrease the analyte elution temperature [116–118].

7. Abbreviations

Ac	*O*-Acetyl
Bu	*O*-Butyryl
CD	Cyclodextrin
CD	Cyclodextrin derivative
GC	Capillary gas chromatography
GC–MS	Capillary gas chromatography/mass spectrometry
GC–IRMS	Capillary gas chromatography–isotope ratio mass spectrometry
EAG	Electroantennogram
ee	Enantiomeric excess
EO	Essential oil
ER	Enantiomeric ratio
Es-GC	Enantioselective capillary gas chromatography
Et	*O*-Ethyl
HS	Head-space
D-HS	Dynamic headspace
HPLC	High-performance liquid chromatography
HPr	*O*-Hydroxypropyl
GC×GC	Two-dimensional gas chromatography
LC–GC	High-performance liquid chromatography–capillary gas chromatography
Me	*O*-Methyl
MCSS	Moving column stream switching
NMR	Nuclear magnetic resonance spectrometry
Pe	*O*-Pentyl
PEG	Polyethyleneglycol, Carbowax
Prep-cGC	Preparative capillary gas chromatography
Prep-pGC	Preparative packed column gas chromatography
Prep-GC×GC	Preparative two-dimensional gas chromatography
Prep-HPLC	Preparative HPLC
SDE	Simultaneous distillation extraction
SIM	Selected ion monitoring
SPME	Solid-phase microextraction
SSD	Sulphur-selective detection
TBS	6-*O*-*tert*-Butyldimethylsilyl
TFA	Trifluoroacetyl
THS	6-*O*-Thexyldimethylsilyl

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