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ENANTIOMER ANALYSIS BY COMPLEXATION GAS CHROMATOGRAPHY

SCOPE, MERITS AND LIMITATIONS

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SUMMARY

The application of complexation gas chromatography in contemporary enantiomer analysis was investigated. Enantiomers were separated without derivatization on Chirametal stationary phases, such as manganese(II), cobalt(II) and nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate], coated on high-resolution glass and fused-silica open-tubular columns. Enantiomeric excesses (ee) up to 99.9% can be precisely determined by complexation gas chromatography. Employing the gas chromatography–mass spectrometry (single ion monitoring) technique, the determination of ee and absolute configurations of solutes, present in complex mixtures, can be performed at the low picogram level. For the first time, pertinent sources of error in the gas chromatographic determination of ee were identified and novel experimental verifications thereof are presented. Interconversion profiles due to inversion of configuration during enantiomer separation by complexation gas chromatography have been detected. Inconsistencies of an empirical quadrant rule, correlating absolute configuration and order of elution of cyclic ethers from nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate], are reported.

INTRODUCTION

The unequivocal determination of enantiomeric compositions and absolute configurations is an important analytical task in research concerned with the synthesis, characterization and use of chiral compounds. The last decade has witnessed a substantial improvement in enantioselective synthesis, *e.g.*, in catalytic asymmetric transformations, asymmetric syntheses, kinetic resolutions and biomimetic reactions[1]. The enantiomeric excess (ee) provides a quantitative criterion for the success of any enantioselective access to non-racemic chiral compounds. The precise determination of enantiomeric compositions may also be necessary in the characterization of natural products, such as flavouring and fragrant materials, or pheromones, in the monitoring of the enantiospecificity of enzymic reactions, in the detection of racemization in "chiral pool" synthesis, in peptide synthesis/hydrolysis and in the study of reaction mechanisms. The availability of reliable and precise techniques for the cor-

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rect determination of ee is therefore of great importance in order to cope with the analytical requirements of contemporary stereochemistry.

The quantitative separation of enantiomers by gas chromatography (GC) on chiral, non-racemic stationary phases[2–5] constitutes a powerful tool for enantiomer analysis, because of its speed, simplicity, reproducibility and sensitivity[6,7]. In complexation GC, an electronically and coordinatively unsaturated transition metal compound is added to the liquid stationary phase. Owing to the fast and reversible chemical interaction between the additive and the substrate, which possesses suitable chemical functionalities for coordination, the separation of structural isomers and isotopomers can be carried out by virtue of chemical selectivity. A fascinating highlight of chemical selectivity is enantioselectivity, which will lead to the discrimination of optical isomers on chiral, non-racemic metal coordination compounds, employed as additives to the stationary phase[8,9]. Thus, racemic ethers, ketones, alcohols, acetals, esters and racemates of other classes of compounds have been quantitatively separated into enantiomers with high-resolution open-tubular columns coated with manganese(II), cobalt(II), or nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1), and related Chirametal stationary phases[10–12]. In contrast to complementary methods of GC enantiomer separation[2,3], substrate derivatization is generally not required in complexation GC, rendering enantiomer analysis by this method very straightforward. The use of complexation GC in enantiomer analysis has been summarized previously[6,7]. Here we report on improvements to the method by employing high-resolution glass and, particularly, fused-silica open-tubular column technology[13]. The scope, merits and limitations of complexation GC for the determination of ee and absolute configurations of volatile chiral compounds are considered in depth.

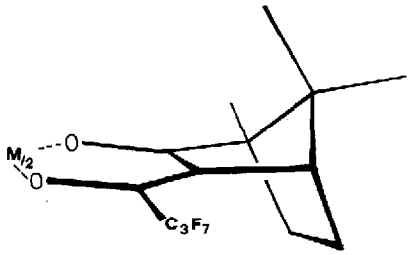
EXPERIMENTAL

Instrumentation

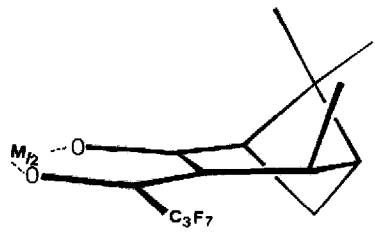
Carlo Erba Fractovap 2101 and 2350 gas chromatographs, equipped with flame ionization detectors and suitable for operation with glass open-tubular columns, were used. A Carlo-Erba HRGC 5300 MEGA gas chromatograph was used with fused-silica open-tubular columns. The carrier gas was nitrogen (caution: hydrogen must not be employed as the carrier gas in complexation GC!). The splitting devices were set at 1:100. In order to avoid overloading, which results in peak tailing and broadening, the instrument was set to its highest sensitivity at a tolerable signal-to-noise ratio.

Open-tubular columns

The preparation of glass and fused-silica open-tubular columns for use in complexation GC has been described in detail previously[13,14,41]. Manganese(II) and nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1) and nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*,2*S*)-pinanone-4-ate] (2) were prepared as described in the literature[11,12,40,41]. Strict adherence to the published procedures is recommended. Chirametal stationary phases prepared and purified by alternative routes may exert different physical and chromatographic properties. For the coating of the columns, only freshly prepared and purified. Chirametal stationary phases should be employed (caution: Chirametal stationary phases should be handled with appropriate care).



1 a : M = Mn
b : M = Co
c : M = Ni



2 : M = Ni

The columns, coated with 1a–c and 2, exhibited long lifetimes when measurements were performed below 90°C. Columns coated with 1c can be operated for extended periods at 100°C and for short times at 130°C. When not in use, the column ends should be kept closed. During operation, the detector should be ventilated into a hood. Contamination of the Chirametal stationary phase with water and oxygen must be avoided.

Up-dated information on the practical use and the current availability of tailor-made capillary columns for chiral complexation chromatography can be obtained from the author on request.

RESULTS AND DISCUSSION

Scope of complexation gas chromatography for enantiomer separation

The use of glass or fused-silica high-resolution open-tubular columns coated with Chirametal stationary phases has been reported previously[13,14]. This development initiated many applications of complexation GC in various fields of enantiomer analysis[15–22]. In Figs. 1–8 some representative chromatograms are shown, featuring the direct, derivatization-free enantiomer separation of different classes of compounds, such as spiroacetals, oxiranes, alcohols, diol acetonides and esters by complexation GC.

The chromatograms vividly demonstrate that the objective of separating enantiomers quantitatively in a short time has been met successfully by high-resolution capillary complexation GC. The highly deactivated state of the columns can be recognized, *e.g.*, from the absence of peak tailing in the chromatograms of selected alcohols (*cf.*, Figs. 5 and 6).

Enantiomer separation by complexation GC is brought about by chemical selectivity. The influence of column temperature, concentration of the Chirametal in the stationary phase, column length, thickness of the coating, pressure drop and nature of the carrier gas on enantiomer separation will obviously be different from their equivalents in conventional, physical partition GC. No systematic study on the influence of various chromatographic parameters and their interdependence on the separation factor of enantiomers is yet available, but some empirical rules have already emerged. While hydrogen interferes with Chirametal stationary phases and must therefore be excluded in complexation GC, no particular improvement in en-

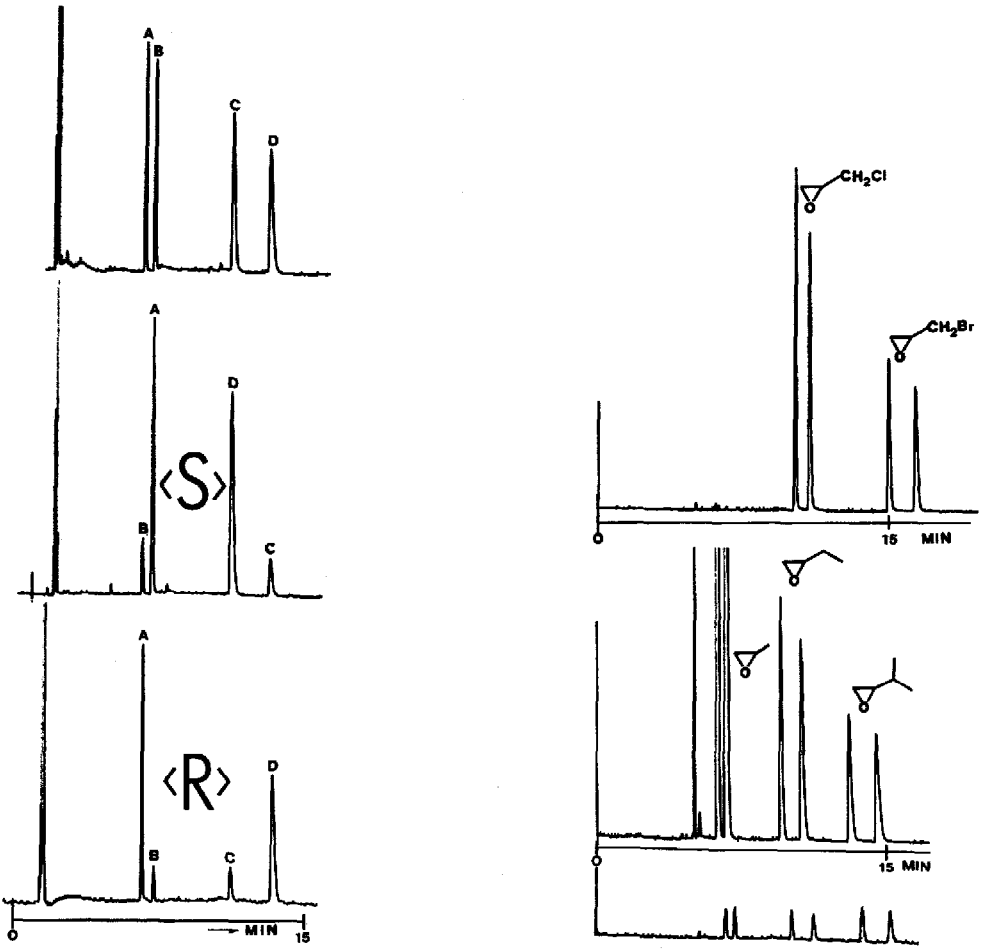


Fig. 1. Enantiomer separation of, and peak inversion for, (*Z*)- and (*E*)-2-ethyl-1,6-dioxaspiro[4.4]nonane ["chalcogran", the principal aggregation pheromone of *Pityogenes chalcographus* (L.)] (3), on 25 m × 0.25 mm I.D. fused-silica capillary columns, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] [(*R*)-1c] and nickel(II) bis[3-(heptafluorobutanoyl)-(1*S*)-camphorate] [(*S*)-1c], respectively, in SE-54 (0.2 μm). Oven temperature, 93°C; inlet pressure, 1 bar nitrogen. Top: racemic 3 on (*R*)-1c. Centre: 3 enriched in the 2*S* enantiomer on (*S*)-1c. Bottom: 3 enriched in the 2*S* enantiomer on (*R*)-1c. Peak assignment: A = (2*S*,5*S*)-3; B = (2*R*,5*R*)-3; C = (2*R*,5*S*)-3; D = (2*S*,5*R*)-3[13].

Fig. 2. Enantiomer separation of simple chiral oxiranes on a 25 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* cobalt(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1b) in SE-54 (0.2 μm). Oven temperature, 55°C; inlet pressure, 1 bar nitrogen. Top: epichlorohydrin [(chloromethyl)oxirane] and epibromohydrin [(bromomethyl)oxirane]. Bottom: methyl-, ethyl- and isopropyloxirane.

antiomer separation is observed when nitrogen, the preferred carrier gas, is replaced with helium. High pressure drops, which allow a faster analysis, are not necessarily detrimental to enantiomer separation. A decrease in the peak resolution, R_s, from 2 to 1 has been observed for the enantiomers of *exo*-brevicomin (4) when the nitrogen inlet pressure was increased from 1.4 to 3.0 bar. The use of short columns may be

Fig. 3. Enantiomer separation of 1-methylcyclohexene oxide (1-methyl-7-oxabicyclo[4.1.0]heptane) on a 10 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.03 *m* manganese(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1a) in SE-54 (0.2 μm). Oven temperature, 50°C; inlet pressure, 0.25 bar nitrogen.

Fig. 4. Enantiomer separation of isopropyl- and *tert.*-butyloxirane on a 10 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* manganese(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1a) in SE-54 (0.2 μm). Oven temperature, 30°C; inlet pressure, 0.25 bar nitrogen.

very beneficial in enantiomer separation, as Figs. 3 and 4 demonstrate. Thus, racemic isopropyloxirane and *tert.*-butyloxirane are simultaneously resolved into enantiomers in 150 s and 1-methylcyclohexene oxide is separated in 5.5 min.

The enantiomer separation of (*Z*)- and (*E*)-2-ethyl-1,6-dioxaspiro[4.4]nonane ["chalcogran", the principal aggregation pheromone of *Pityogenes chalcographus*

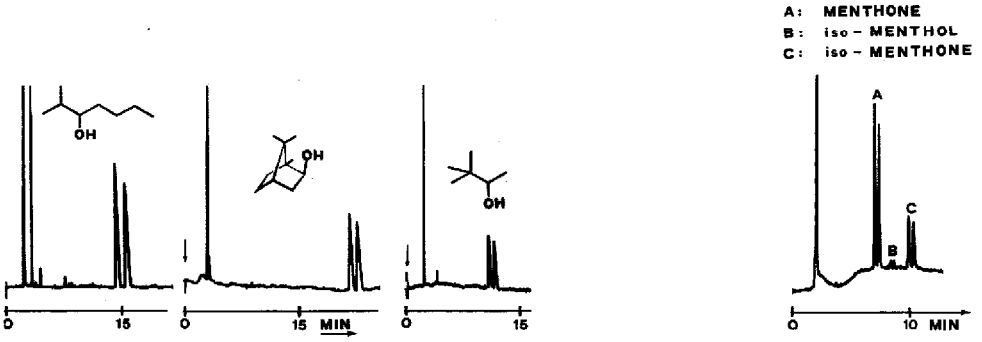


Fig. 5. Enantiomer separation of underivatized alcohols on a 30 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in SE-54 (0.2 μm). Left: 2-methyl-3-hydroxyheptane (85°C; 0.8 bar nitrogen). Centre: isoborneol (75°C; 0.75 bar nitrogen). Right: *tert.*-butylmethylcarbinol (70°C; 0.9 bar nitrogen).

Fig. 6. Simultaneous enantiomer separation of menthone, isomenthol and isomenthone on a 25 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in SE-54 (0.2 μm). Oven temperature, 120°C; inlet pressure, 1 bar nitrogen.

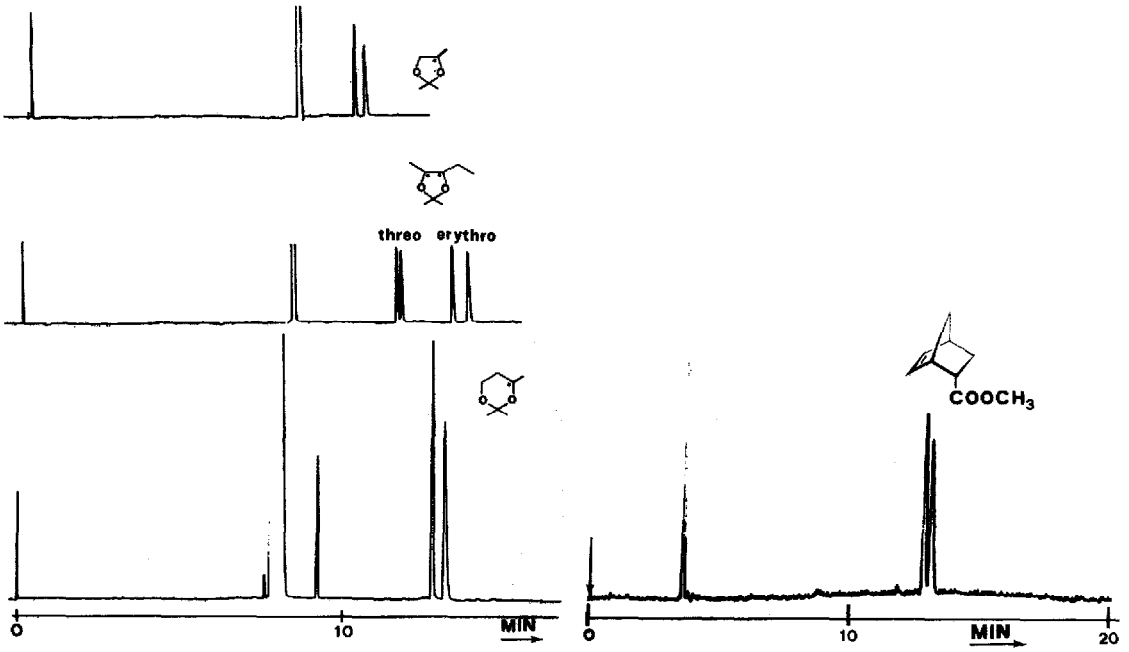
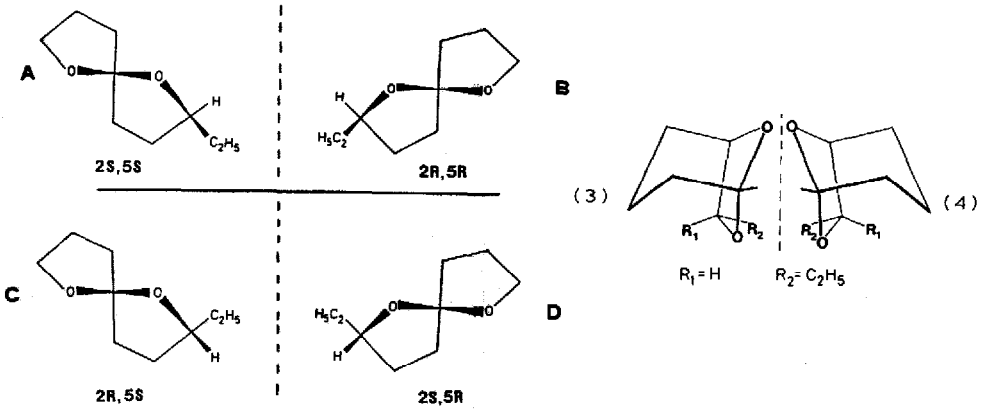


Fig. 7. Enantiomer separation of 1,2-propanediol acetonide, *threo*- and *erythro*-2,3-pentanediol acetonide and 1,3-butanediol acetonide on a 50 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*,2*S*)-pinanone-4-ate] (2) in SE-54 (0.2 μm). Oven temperature, 80°C; inlet pressure, 0.7 bar nitrogen.

Fig. 8. Enantiomer separation of methyl norborn-5-ene-2-*endo*-carboxylate on a 25 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*S*)-camphorate] (1c) in SE-54 (0.2 μm). Oven temperature, 110°C; inlet pressure, 0.4 bar nitrogen.

(L.)] (3), depicted in Fig. 1, has also been performed in the gas chromatography–mass spectrometry (single ion monitoring) [GC–MS(SIM)]mode[23] (*cf.*, Fig. 9). No bleeding of the Chirametal stationary phase nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) has been observed at 100°C, as judged from the absence of



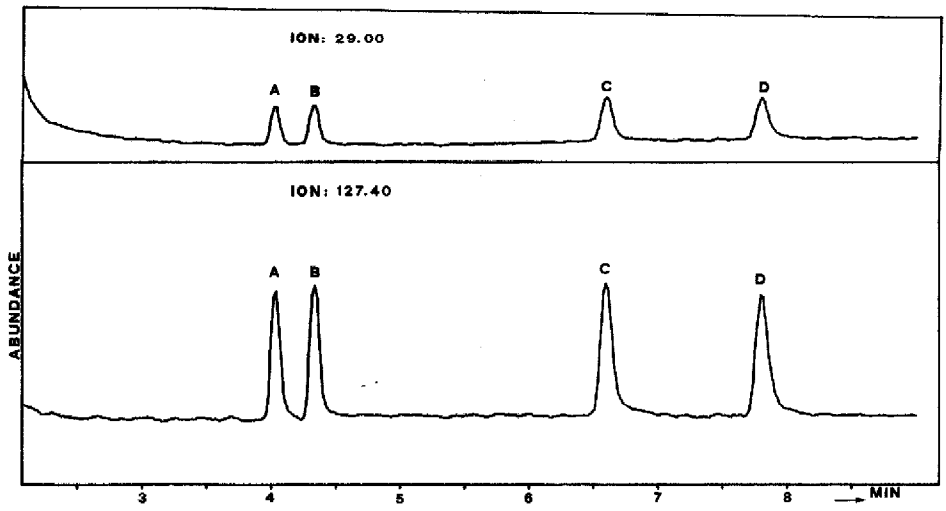


Fig. 9. GC–MS(SIM) (HP 5970) of 20 ppm (in *n*-hexane) of (*Z*)- and (*E*)-2-ethyl-1,6-dioxaspiro[4.4]nonane ("chalcogran") (3) (*cf.*, Fig. 1) on a 25 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in SE-54 (0.2 μm). Oven temperature, 100°C. Top, ion mass 29 emu; bottom, ion mass 127.4 emu[23].

additional peaks in the mass spectrum. The peak resolution and peak symmetry improved as a sample of 3 (1% in *n*-hexane) was diluted to 250 and 10 ppm. This result is in keeping with the general observation that complexation GC is particularly sensitive to overloading conditions resulting in peak broadening and tailing. The comparison between the sampling of 250 and 10 ppm of 3 also revealed that the Chirametal stationary phase responded linearly to all four configurational isomers and, therefore, quantitation was not biased by irreversible absorption of the sample[23]. From the GC–MS(SIM) experiment with the masses 29 and 127 a detection limit of the configurational isomers of 3 of 0.2–1 pg on an absolute scale on a 25 m × 0.25 mm I.D. open-tubular column was estimated[23].

As 3 can be separated into four configurational isomers without chemical manipulation, the stereochemistry of the natural pheromone could be easily determined from complex biological matrices via GC–MS(SIM)[13].

Merits and limitations of complexation gas chromatography in enantiomer analysis

In contemporary enantiomer analysis, the method of choice must be capable of identifying minute amounts of an enantiomeric impurity. It should be recalled that in an asymmetric reaction a free-enthalpy difference of activation of only $\Delta(\Delta G^{\ddagger})$ = 4.0 kcal/mol (291 K) between the diastereomeric transition states will lead to the formation of as little as 0.1% of the antipode (ee = 99.8%)[6].

The GC separation of chiral molecules constitutes a powerful tool for modern high-precision enantiomer analysis[7]. The method is particularly successful in two borderline cases, *viz.*, in the detection of minute deviations from a true racemic mixture (*e.g.*, to measure ee ≠ 0% in experiments devoted to the amplification of optical activity under abiotic conditions) and for the determination of traces of enantiomeric

PEAK#	AREA%	RT	AREA	BC
1	1.827	1.5	1109	01
2	49.487	4.33	53456	01
3	49.487	4.98	53456	01
TOTAL	100.		108021	

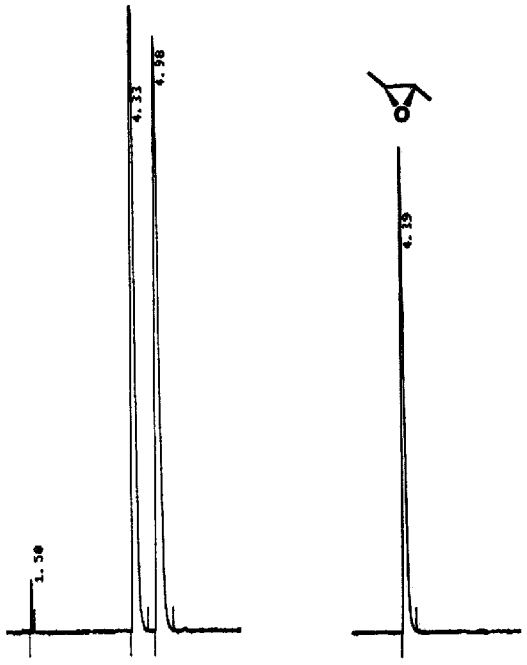


Fig. 10. Enantiomer separation of *trans*-2,3-dimethyloxirane on a 25 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.15 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in SE-54 (0.2 μm). Oven temperature, 80°C. Integration with Spectra-Physics SP 4100 instrument. Left: racemic *trans*-2,3-dimethyloxirane (peak areas: first, 53456; second, 53456). Right: *trans*-(2*R*,3*R*)-2,3-dimethyloxirane (only one peak is identified)[24].

impurities (*e.g.*, to measure ee ≈ 100% in "total" asymmetric synthesis). The results in Fig. 10 demonstrate that the challenge of determining extraordinary figures for ee can be met by the experiment[24]. Thus, integration of the enantiomeric elutions of a truly racemic specimen (because it is synthesized in a achiral environment) of *trans*-2,3-dimethyloxirane showed an unprecedented agreement of the peak areas, that is (arbitrary scale): 5345^6 *vs.* 5345^6, ee = 0.000%. The corresponding optically active solute, (2*R*,3*R*)-2,3-dimethyloxirane, prepared without racemization from enantiomerically pure (2*R*,3*R*)-2,3-butanediol[25], gave only one peak with no detectable trace of the antipode within the detection limit of the instrumental set-up.

In contrast to other methods, such as NMR spectroscopy and polarimetry[26], the determination of 0.1% of antipodal impurities by GC presents no difficulty, provided that the peak resolution is high (for enantiomer separations exhibiting small peak resolutions, *R*, a multi-scanning procedure has been devised for chromatographic trace analysis[27]). In Fig. 11 the high enantiomeric purity of ee = 99.85% for a

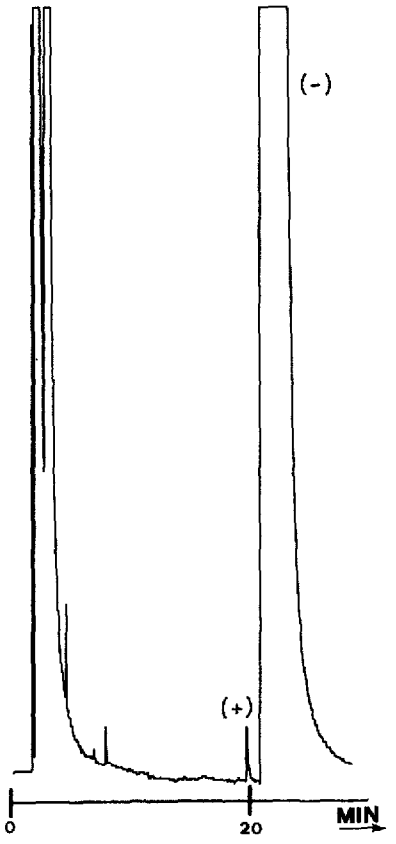


Fig. 11. Determination of ee of (1*S*,7*S*)-(−)-*exo*-brevicomin (4), synthesized by Mori and Seu[28], on a 50 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*,2*S*)-pinanone-4-ate] (2) in SE-54 (0.2 μm); ee = 99.85%; digitized integration (Shimadzu C-R3A) of the antipodal impurity, 0.076%[7].

synthetic sample of the pheromone (1*S*,7*S*)-(−)-*exo*-brevicomin (4)[28], determined on nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*,2*S*)-pinanone-4-ate], (2), is shown. As the antipodal impurity is eluted before the main enantiomeric fraction, peak integration presents no difficulty, and it is obvious that even greater enantiomeric purities may be identified in the future by complexation GC. The availability of Chirametal stationary phases of opposite chirality greatly facilitates enantiomer analysis because the elution order of the chiral impurity may be inverted if the minor enantiomer is eluted as the second peak and will be partially lost in the tail of the major eluate. Most terpene ketonates used for the synthesis of Chirametal stationary phases are available in both enantiomeric forms, *e.g.*, camphor, carvone, menthone and 4-pinanone. Chirametal stationary phases with opposite chiralities were first used in complexation GC to differentiate a true enantiomer separation from accidental 1:1 separations of achiral isomers[9]. Clearly, peak inversion on chromatography on "mirror-image" stationary phases can only occur for an enantiomeric mixture when the "image" and its "mirror image" are non-superimposable. In Fig. 1 (centre, bottom)

a characteristic example of a peak inversion is illustrated for 3, chirally labelled *S* on carbon atom 2 and chromatographed on both nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (*R*-1c) and nickel(II) bis[3-(heptafluorobutanoyl)-(1*S*)-camphorate] (*S*-1c). Peak reversal is clearly demonstrated for the *Z*-(A,B) and *E*-(C,D) enantiomers, respectively.

Sources of error in the determination of enantiomeric compositions (ee) by gas chromatography

Despite the great success of GC in enantiomer analysis, it is now prudent to discuss potential errors in the determination of ee. The following sources of error in the determination of ee can be discerned[7]: (i) decomposition of the solute on achiral parts of the column (the enantiomer which spends a longer time in the column will be lost preferentially, causing an error in ee); (ii) decomposition of the solute on the chiral stationary phase (this chemical change may proceed enantioselectively, causing the preferential loss of one antipode); (iii) contamination of peaks with impurities, spuriously increasing peak areas; (iv) fractionation of enantiomers by the "EE" effect during sample manipulation (the scalar physical properties of enantiomers may vary with the enantiomeric composition); (v) "enantiomerization" of configurationally labile solutes, producing peak distortions due to inversion of configuration during enantiomer separation; (vi) non-linear detector response; (vii) peak distortions caused by inadequate instrumentation.

A serious error in the determination of enantiomeric compositions may be caused by preferential decomposition of one enantiomer during its residence time in the column. Decomposition of the solute on achiral parts of the column will have the effect that the antipode which spends a longer time in the column will be lost preferentially and, as a consequence, ee will be biased in favour of the first-eluted enantiomer. This effect can be recognized by a deviation from the expected 1:1 ratio for a racemic mixture, whereby only the area of the second peak will be diminished.

Decomposition of the solute on the chiral stationary phase will also have the effect that the antipode which spends a longer time in the column will be lost preferentially and ee will be biased in favour of the first-eluted enantiomer. Again, this effect is recognized by a deviation from the 1:1 ratio for a racemic mixture whereby the area of the second peak will be diminished.

A dramatic example is depicted in Fig. 12. On complexation GC of racemic epichlorohydrin on cobalt(II) bis[3-(heptafluorobutanoyl)-(1*R*)-comphorate] (1b) at 60°C, a striking deviation from the expected 1:1 ratio is observed, which can readily be recognized from the different peak areas where the second peak is diminished[29]. Other examples of deviations from the expected 1:1 ratio have been observed in complexation GC of racemic 1-methylcyclopentene oxide (1-methyl-6-oxabicyclo[3.1.0]hexane) and of racemic 1-methylcyclohexene oxide (1-methyl-7-oxabicyclo[4.1.0]heptane) on nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) at 60 and 80°C, respectively (*cf.*, Fig. 13)[30], whereby the great differences of the retention times caused by an exceedingly high separation factor α should also be noted. The deviation from the expected 1:1 ratio is not observed on manganese(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1a) and, therefore, this Chirametal stationary phase should be employed for the screening of ee of 1-methylcyclopentene oxide and 1-methylcyclohexene oxide (*cf.*, Fig. 3). In general, the error in ee due to decom-

position of the solute can be reduced if the difference in the residence time in the column is minimized for both enantiomers. This may be achieved by using short columns, high pressure drops and a Chirametal stationary phase that exhibits only small separation factors.

It will not be possible to tell whether decomposition of the solute occurs on achiral or chiral parts of the column although, in the examples cited above, the epoxide ring opening may be expected to take place on the metal ion. When the decomposition of the solute is mediated by the Chirametal stationary phase in an enantioselective fashion in favour of the first-eluted enantiomer, the area of the first peak will necessarily be diminished. It occurred to us that such a rare phenomenon could be verified experimentally[29]. The solute was again a strained epoxide which is prone to ring opening. According to Fig. 14, complexation GC of racemic *trans*-2-methyl-3-phenyloxirane on nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) at 60°C gave rise to a deviation from the expected 1:1 ratio, where the area of the first peak was clearly diminished[29]. With increasing temperature this effect became dramatic and a new broad elution zone, probably created by unidentified reaction products, was built up after the elution of the sharp peaks. It should be noted that this deviation from the expected 1:1 ratio was not observed with either the racemic *cis*-isomer or racemic trimethylphenyloxirane[29].

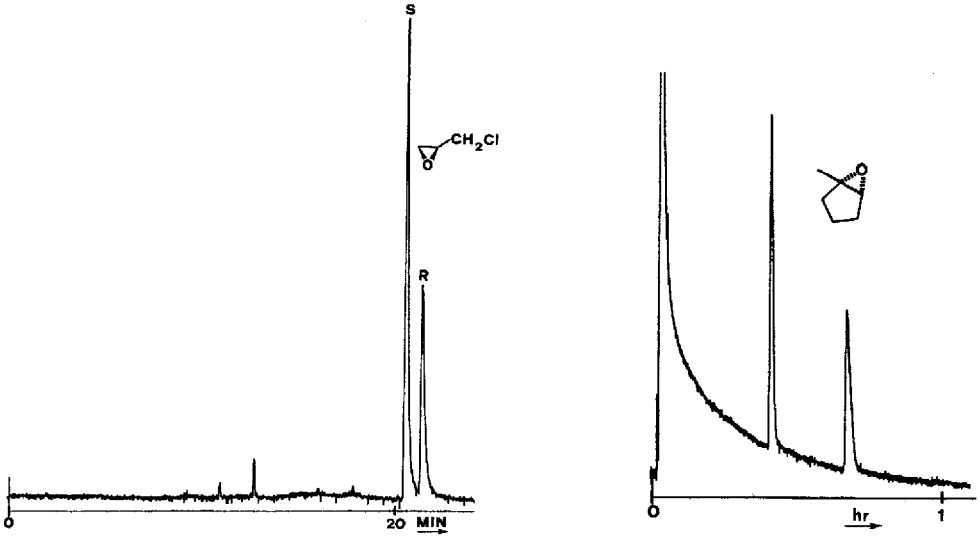


Fig. 12. Deviation from the expected 1:1 ratio on enantiomer separation of racemic epichlorohydrin [(chloromethyl)oxirane] on a 63 m × 0.25 mm I.D. glass capillary column coated with 0.1 *m* cobalt(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1b) in OV-101. Oven temperature, 60°C; inlet pressure, 1 bar nitrogen[29].

Fig. 13. Deviation from the expected 1:1 ratio on enantiomer separation of racemic 1-methylcyclopentene oxide (1-methyl-6-oxabicyclo[3.1.0]hexane) on a 25 m × 0.25 mm I.D. glass capillary column coated with 0.08 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in OV-101. Oven temperature, 60°C; inlet pressure, 1 bar nitrogen[30].

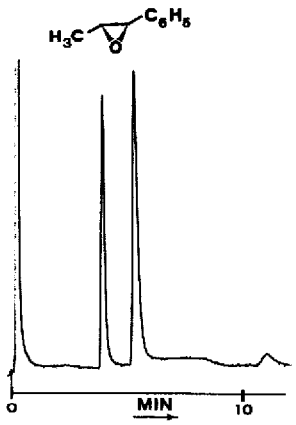


Fig. 14. Deviation from the expected 1:1 ratio on enantiomer separation of racemic 2-methyl-3-phenyloxirane on a 10 m × 0.25 mm I.D. glass capillary column coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in OV-101. Oven temperature, 80°C; inlet pressure, 1 bar nitrogen[29].

A trivial origin of a deviation from the expected 1:1 ratio of a racemic mixture observed in GC enantiomer separation is the peak contamination with impurities, spuriously increasing peak areas[31].

Errors due to (i), (ii) and (iii) above may be recognized by conducting the ee determination with stationary phases of opposite chirality, as has been suggested previously[7,9,31,32]. Errors due to (iii) may also be recognized and eliminated, by changing the chromatographic parameters, such as temperature or carrier gas flow-rate. To be on the safe side, verification of the expected 1:1 ratio on GC enantiomer separation is strongly recommended in enantiomer analysis.

Deviations from the original ee of a mixture of enantiomers may, in principle, arise during sample manipulation via self-association of the enantiomers to diastereomeric dimers with like or unlike configuration exhibiting distinct physical properties (the "EE-effect"[33]). This effect, in which "the relative amounts of two enantiomers induce an observable difference between them"[33], may well lead to accidental fractionation of enantiomers during work-up, isolation or chromatographic separation. For example, it was demonstrated in three independent reports that the enantiomeric composition had changed during the chromatography of an enantiomerically enriched mixture on an achiral stationary phase when sample recovery was incomplete[33–35].

This effect may be rationalized (in a very oversimplified manner) by assuming that the enantiomers in excess do function as a chiral environment for the enantiomer separation of the residual racemic mixture. Whether or not an "EE-effect" is important in sampling procedures with splitting devices in GC is open to discussion. Consider the determination of 0.1% of an antipode D in a mixture with 99.9% L. Let us assume that D will preferentially combine with the excess of L to give DL, while L will combine with L to give LL. If LD is much more volatile than LL, the former will be preferentially lost on evaporation in the injector through the splitting device and the ee of L will therefore be overestimated. Clearly, on-column injection will eliminate this source of error.

The detector response to a racemic mixture of enantiomers is strictly 1:1, as enantiomers cannot be distinguished in an achiral environment. (Incidentally, a racemic mixture, prepared in an achiral environment, represents an ideal equimolar mixture of components with identical (non-chiroptical) physical properties which may be employed to verify the precision of integration facilities). In enantiomer analysis, a linear detector response is indispensable. Thus, for the correct determination of, *e.g.*, 0.1% of an enantiomeric impurity, linearity within a concentration range of three orders of magnitude is required. It is generally accepted that the flame ionization detector does fulfil this requirement, but verification of the linear detector response by dilution experiments is recommended.

Selected chiral solutes possessing configurationally labile chiral centres, such as spiroacetals or aziridines[12], may be prone to enantiomerization (change of configuration) during enantiomer separation by complexation GC, an effect which gives rise to characteristic chromatographic interconversion profiles[12]. A detailed peak-shape analysis of the so-called "peak coalescence of the second kind" has been presented and kinetic activation parameters of enantiomerization have been calculated[36] (*cf.*, Fig. 15). This effect can be recognized by the appearance of an overlapping zone ("plateau"), caused by inverted molecules between the two terminal peaks arising from non-inverted molecules. The determination of ee will, of course, be biased by such an effect. An instructive example of interconversion profiles as a result of a configurational change at the spiro centre in 3 is shown in Fig. 16. Thus, the isomer A (2*S*,5*R*) is converted to its epimer, D (2*S*,5*S*), while the enantiomer of A, *i.e.*, B (2*R*,5*S*), is converted to its epimer, C (2*R*,5*R*). As this epimerization is obviously catalysed by the Chirametal stationary phase in an enantioselective way, the enantiomeric ratio of the terminal peaks may, in principle, be different from unity. The characteristic interconversion profile depicted in Fig. 16 is created by the superpo-

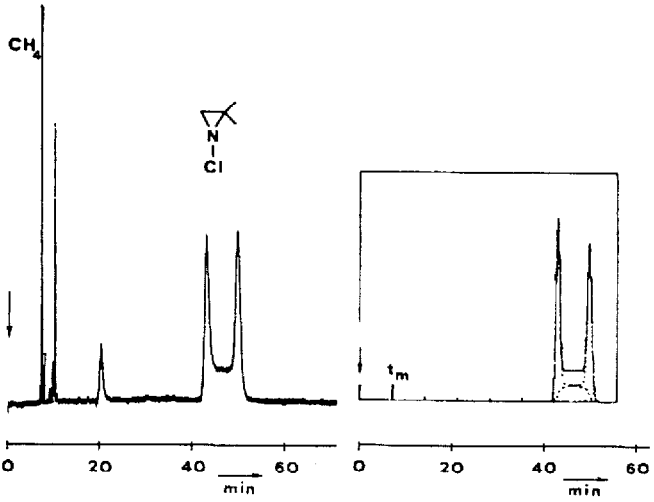


Fig. 15. "Peak coalescence of the second kind"[12] due to inversion of configuration of 1-chloro-2,2-dimethylaziridine on a 100 m × 0.5 mm I.D. nickel capillary column coated with 0.133 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in squalane. Oven temperature, 60°C; inlet pressure, 2.8 ml/min nitrogen. Left, experimental chromatogram[12]; right, calculated chromatogram[36].

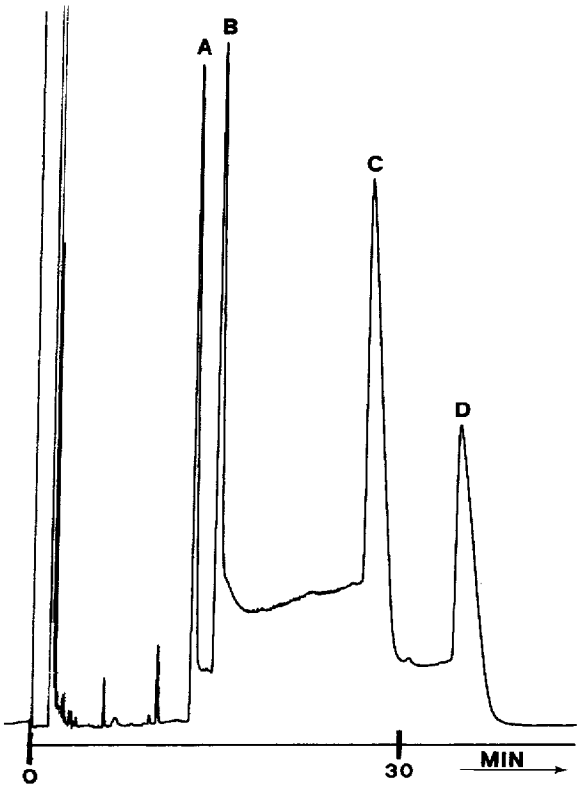


Fig. 16. "Peak coalescence of the second kind"[12] due to inversion of configuration of (*Z*)- and (*E*)-2-ethyl-1,6-dioxaspiro[4.4]nonane ("chalcogran") (3) on a 25 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in OV-101 (0.2 μm). Oven temperature, 93°C; inlet pressure, 0.4 bar nitrogen. For peak assignment see Fig. 1.

sition of two plateaux to give a "double-plateau". A particularly complex interconversion profile characterized by a grossly distorted double plateau is shown in Fig. 17. It is assumed that the phenomenon is probably due to a particular specimen of nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) used as a Chirametal stationary phase which might have undergone transformations to active species during storage.

Peak distortions having their origin in instrumental inadequacies may also vitiate enantiomer analysis by GC. Owing to the chemical nature of interactions in complexation GC and to the low capacity of the Chirametal stationary phases, the elution from the column is extremely sensitive to temperature gradients in the column compartment of the gas chromatograph. Inadequate oven heating will cause thermal peak splitting when open-tubular columns made from vitreous materials of low thermal capacity are used in complexation GC. These periodic fluctuations in the chromatographic eluates are predominant at high retentions and are recognized by the co-called "christmas-tree" shape of the chromatographic peaks[37,38]. This effect is demonstrated in Fig. 18 (left). Wrapping of the fused-silica column with aluminium

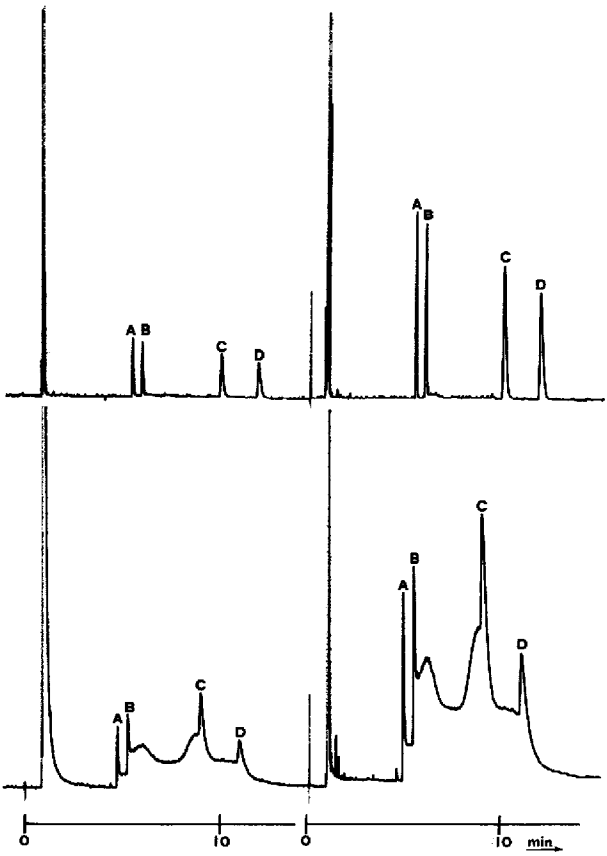


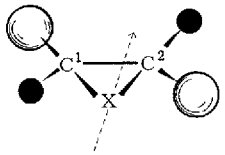
Fig. 17. "Peak coalescence of the second kind"[12] accompanied by enhanced peak distortion due to inversion of configuration of (*Z*)- and (*E*)-2-ethyl-1,6-dioxaspiro[4.4]nonane ("chalcogran") (3) on a 25 m × 0.25 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in OV-101 (0.2 μm). Oven temperature, 93°C; inlet pressure, 0.4 bar nitrogen. Top, standard column with no recognizable effect; bottom, column producing pronounced interconversion profiles. For peak assignment, see Fig. 1.

foil will increase the heat capacity of the column and thus reduce the peak distortion (Fig. 18, right). Even with contemporary, highly sophisticated commercial gas chromatographs this effect can readily be observed in complexation GC[19].

Correlation of absolute configuration and order of elution in complexation gas chromatography

The determination of absolute configurations is an important task in enantiomer analysis. Absolute configurations of minute amounts of chiral solutes may be determined free of chiroptic evidence by GC by means of simultaneous injection of reference compounds with known stereochemistry. Thus, complexation GC has been successfully employed to determine the stereochemistry of pheromones[15] and of flavouring materials[16] without the need for solute derivatization and chemical manipulations.

In complexation GC, a consistent relationship between the order of elution and absolute configuration of homologous compounds has been observed[39]. For instance, (*S*)-methyloxirane and (2*S*,3*S*)-2,3-dimethyloxirane exhibited a stronger interaction and were therefore eluted as the second peak on nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c), obtained from natural (+)-*d*-(1*R*)-camphor[39]. This observation led to the formulation of a quadrant rule, which empirically predicts the elution order of alkyl-substituted three-membered heterocycles from a GC column, containing 1c derived from (1*R*)-camphor.



X = NH, O, S

Thus, when the heterocycle is viewed from the heteroatom X in the direction of the horizontal C–C bond, the absolute configuration of the enantiomer eluted as the second peak from (1*R*)-1c is that in which the bulkier group(s) is (are) situated on the upper left at C_1 and/or the lower right at C_2. It was later confirmed that the enantiomers of monoalkyl-substituted oxiranes [alkyl = methyl, ethyl, isopropyl, *sec.*-butyl (two isomers), *tert.*-butyl], *trans*-2,3-dialkyl-substituted oxiranes (dialkyl = dimethyl and ethylmethyl), trimethyloxirane, (chloromethyl)oxirane (epichlorohydrin) and *rac*-dioxirane were eluted from (1*R*)-1c in the order predicted by the quadrant rule[12,40]. The validity of the quadrant rule has also been confirmed for monoalkyl-substituted oxiranes (alkyl = methyl, ethyl, isopropyl, *tert.*-butyl), *trans*-2,3-dimethyloxirane and trimethyloxirane and manganese(II) and cobalt(II)

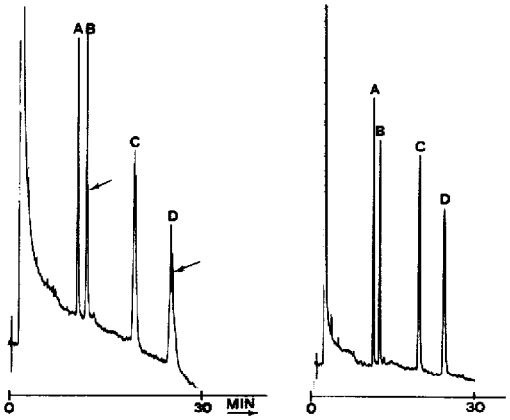


Fig. 18. "Christmas-tree-effect" (*cf.*, arrows in chromatogram on the left), as revealed by thermal peak splitting due to temperature fluctuations in the oven, observed in the enantiomer separation of (*Z*)- and (*E*)-2-ethyl-1,6-dioxaspiro[4.4]nonane ("chalcogran") (3) on a 25 m × 0.32 mm I.D. fused-silica capillary column, coated with 0.1 *m* nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) in OV-101 (0.5 μm). Oven temperature, 93°C; inlet pressure, 1 bar nitrogen. Gas chromatograph, Carlo Erba Fractovap 2101, performance without (left) and with (right) wrapping of the fused-silica capillary column with aluminium foil. For peak assignment, see Fig. 1.

bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1a and 1b) derived from natural (1*R*)-camphor[12]. The quadrant rule is also valid for *trans*-1-chloro-2-methylaziridine and methylthiirane, eluted from nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c). However, a remarkable exception to the rule has been found for *tert.*-butylthiirane, *trans*-2,3-dialkylthiiranes (alkyl = dimethyl and ethylmethyl) and for trimethylthiirane, which showed an inverse elution order on nickel(II) bis [3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c) as compared with the corresponding oxiranes[12].

Attempts to extend the quadrant rule to cyclic ethers with four-, five- and six-membered rings led to ambiguous results. Thus, while (*S*)-methyloxirane, (*S*)-2-methyloxetane and (*S*)-2-methyltetrahydrofuran were eluted as the second peak from manganese(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1a), as predicted by the quadrant rule, (*S*)-methyloxirane and (*S*)-2-methyloxetane were also eluted as the second peak, but (*S*)-2-methyltetrahydrofuran and (*S*)-2-methyltetrahydropyran were eluted as the first peak from nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c)[40]. It is noteworthy that the elution orders of the enantiomers of 2-methyltetrahydrofuran on manganese(II) and nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1a and 1c) are different. Still more puzzling is the observation of a change in the elution order as the result of constitutional isomerism or homologization of the solute. Thus, (*S*)-2-methyltetrahydrofuran and (*S*)-3-methyltetrahydrofuran, which were obtained by hydrogenation from the same enantiomerically enriched precursor, homofuran (2-oxabicyclo[3.1.0]-2-hexene)[17], showed an inverse retention behaviour on manganese(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1a), *i.e.*, the former was eluted as the second peak while the latter was eluted as the first peak. Similarly, (*S*)-2-methyltetrahydrofuran was eluted as the first peak while (*S*)-2-ethyltetrahydrofuran was eluted as the second peak from nickel(II) bis[3-(heptafluorobutanoyl)-(1*R*)-camphorate] (1c)[18].

A remarkable change of the sign of the enantioselectivity, caused by a variation in the perfluoroacyl part of manganese(II) bis[3-(perfluoroacyl)-(1*R*)-camphorate], has been observed with monoalkyl-substituted oxiranes (alkyl = ethyl, isopropyl, *tert.*-butyl), *trans*-2,3-dimethyloxirane, trimethyloxirane and 2-methyloxetane. Whereas on manganese(II) bis[3-(trifluoroacetyl)-, -(pentafluorobutanoyl)- and -(perfluorooctanoyl)-(1*R*)-camphorate] the *S*-enantiomers are eluted as the second peak, in agreement with the quadrant rule, the reverse is true for manganese(II) bis[3-(pentafluorobenzoyl)-(1*R*)-camphorate], *i.e.*, all the *R*-antipodes are eluted as the second fraction[41]. The inconsistency of the quadrant rule suggests that the sign of the enantioselectivity is a very sensitive function of subtle changes in the solute and the composition of the Chirametal stationary phase.

The above results imply that predictions of absolute configuration from retention behaviour for homologous compounds may be ambiguous. Thus, in complexation GC absolute configurations should preferably be assigned by direct evidence, *i.e.*, by simultaneous injection of reference compounds with known chirality, rather than by indirect evidence, *i.e.*, by correlation with the elution order of homologous compounds, which may not be free from pitfalls.

CONCLUSIONS

Enantiomer separation by complexation GC represents a highly efficient and precise technique for the direct determination of the enantiomeric composition (ee) of volatile chiral solutes. Inherent sources of error can be easily eliminated by the choice of suitable experimental conditions. A serious limitation to the method is the requirement for solute volatility, thermal stability and quantitative resolvability. The development of improved thermostable and enantioselective Chirametal stationary phases will probably extend the scope of complexation GC in enantiomer analysis in the future.

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