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USE OF GLASS AND FUSED-SILICA OPEN TUBULAR COLUMNS FOR THE SEPARATION OF STRUCTURAL, CONFIGURATIONAL AND OPTICAL ISOMERS BY SELECTIVE COMPLEXATION GAS CHROMATOGRAPHY

V. SCHURIG* and R. WEBER
Institut für Organische Chemie, Universität Tübingen, D-74 Tübingen (F.R.G.)

SUMMARY

The use of glass and fused-silica open tubular columns for selective separation of structural, configurational and optical isomers (enantiomers) on resolving metal chelates by "complexation gas chromatography" is described. The limitations of metal capillaries and the advantages of glass and fused-silica open tubular columns are discussed and verified by experiments. Novel and highly efficient separations of underivatized enantiomers and diastereomers on chiral metal chelates are demonstrated, and the direct screening of complex biological extracts for the enantiomeric composition of a spiroketal pheromone (Chalcogran) is described.

INTRODUCTION

The introduction of chemical selectivity into the gas chromatographic (GC) separation process by deliberately adding (chiral) metal coordination compounds to the stationary liquid phase[1–5] constitutes a highly promising strategy for the quantitative separation of compounds possessing only subtle differences in structure or configuration, such as constitutional and geometrical isomers, diastereomers, enantiomers (optical isomers) and isotopomers[6]. This methodology, which relies on the fast and reversible interaction between solute and transition-metal additive (and, therefore, is called complexation gas chromatography[7]) would gain additional momentum if the inherent chemical selectivity of the separation process, which can be widely adjusted by the proper choice of appropriate metal chelates, could be combined with the high sensitivity and efficiency of contemporary glass and fused-silica capillary column technology. By this complementary approach not only isomers, which have resisted separation thus far, but also overlapping elution profiles frequently encountered in the analysis of complex multi-component mixtures, may be resolved.

We have previously demonstrated that dicarbonyl rhodium(I) 3-(trifluoroacetyl)-1*R*-camphorate, (1), when dissolved in squalane ($C_{30}H_{62}$) and coated onto a 200-m stainless-steel open tubular column efficiently separates isomeric butenes[8], the isotopomers $C_2H_{4-n}D_n$ of ethylene[9] and the optical isomers of 3-methylcyclopen-

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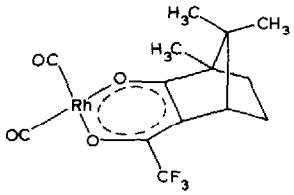
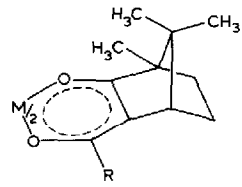
tene[10]. The last of these results represented the first example of enantiomer separation employing chiral metal complexes in GC and it proved that solute–solvent interactions through coordination can have the necessary kinetic and thermodynamic parameters to lead to chiral recognition[11].

Though the scope of selective olefin separations is still limited owing to the low thermal stability of the rhodium complex (1)[6], the method of enantiomer separation by complexation GC has gained considerable practical significance following reports from this laboratory of the use of the thermally stable bis-3-(perfluoroacyl)-1*R*-camphorates of manganese(II), cobalt(II) and nickel(II) (2) (dissolved in squalane and coated onto metal capillary columns) for the first separation of a number of classes of racemic compounds, *e.g.* alkyl-substituted cyclic ethers and thioethers[12–14], N-chloroaziridines[14,15], pheromone acetals[16–19], alcohols and ketones[15,20]. Because these enantiomers are usually baseline separated and, significantly, the racemates need not be derivatized, various useful applications associated with precise determinations of enantiomeric compositions have been reported, *e.g.*, in the field of enantiomeric synthesis[21].



(1)



(2a) M = Mn , R = C_3F_7
(2b) M = Co , R = C_3F_7
(2c) M = Ni , R = CF_3
(2d) M = Ni , R = C_3F_7

Heretofore, essentially metal capillary columns have been used in high-resolution complexation GC. They were dynamically coated with solutions of the metal chelates in squalane[6] and typically showed coating efficiencies[22] (h_{theor}/h_{exp}) of less than 50% (capacity factor, $k' = 9$, *n*-decane). Hence, for high-resolution capillaries (> 100,000 theoretical plates) column lengths up to 200 m were required[6]. Owing to the low operating temperatures (30–80°C) the retention times were long and consequently the sensitivity was low.

Since 1980, following some unsuccessful experiments[6,23], we have been using glass and fused-silica open tubular columns in complexation GC. In 1981 Ôi *et al.*[24] reported the enantiomer separation of alcohols, amino alcohols and amines on glass capillaries coated with solutions of chiral copper(II) Schiff-base complexes in OV-101. However, their separations suffered from the same disadvantages as were described for metal capillaries.

Our efforts have resulted in four preliminary accounts dealing with the quantitative separation of enantiomers[25] and isotopomers[26] using high-resolution glass capillary technology, and its application to the determination of enantiomeric compositions of pheromone acetals present in complex mixtures of biological sources by complexation GC combined with "selected ion" mass spectrometry[27,28]. This paper gives details of the preparation of chemically treated glass and fused-silica open tubular columns amenable to smooth coating with solutions of appropriate resolving metal chelates in silicon liquids, and describes novel examples of direct enantiomer and diastereomer separations by high-resolution complexation GC.

EXPERIMENTAL

Open tubular columns

Duran glass tubing (Schott Ruhrglas, Mainz, F.R.G.) is drawn to capillaries of *ca.* 0.3 mm I.D. using a Hupe and Busch glass-drawing machine. Prior to drawing, the tubes are rinsed with chromosulphuric acid, water and methanol and then dried. Fused-silica capillaries are supplied by Machery, Nagel & Co., Düren, F.R.G.

Column preparation

The following sections give representative procedures for column pre-treatment.

Acid leaching and rinsing[29]. The Duran glass capillary is almost completely filled with aqueous 6 *N* hydrochloric acid, sealed under vacuum, heated to 140–150°C and left overnight. The ends are opened and the capillary is rinsed with approximately three column volumes of 0.01 *N* hydrochloric acid and 1 ml of methanol. Finally, the column is dried at 200°C under a stream of dry nitrogen.

Hydrothermal treatment of fused-silica capillaries[30]. This procedure is a slight modification of the acid leaching for glass capillaries. The capillary is almost completely filled with 6 *N* hydrochloric acid, sealed under vacuum with a micro torch (butane–oxygen), heated to 110°C and left for 3 h. The ends are opened and the column is rinsed with 0.01 *N* hydrochloric acid and then carefully dried under a stream of dry nitrogen.

Deactivation with Carbowax 20M[31]. A glass tube, packed with 20% Carbowax 20M coated on Chromosorb W AW DMCS, is inserted into the hot zone of the injection port. The injector is heated to 280°C, while the oven is maintained at 250°C. The volatiles from the pre-column are allowed to bleed into the capillary for 12 h at a nitrogen flow-rate of 1 ml/min.

Coating. After deactivation the columns are statically coated with 0.3–0.5% solutions of the stationary phase, consisting of the metal chelate and the required amount* of silicon liquid, in *n*-pentane. Prior to installation, *ca.* 10 cm of both column ends are rinsed with methylene chloride and dynamically coated with a 1% solution of SE-30 in *n*-pentane.

Instrumentation

Carlo-Erba gas chromatographs, Fractovap 2101 and 2350, equipped with flame ionization detectors and suitable for operation with open tubular columns were used. The carrier gases were high-purity-grade nitrogen and helium.

Reagents

The chiral metal chelates were prepared as described earlier[13,14]. Silicone phases were supplied by Chrompack, Berlin, F.R.G.

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* For convenience the concentration is referred to the molality scale, *m* (ref. 5).

RESULTS AND DISCUSSION

Metal capillaries

It has become apparent that the surface properties of metal tubings differ not only from manufacturer to manufacturer but also from batch to batch[32–34]. In most cases the column wall possesses a high adsorptivity, especially towards polar compounds. This may have deleterious effects on the chromatographic peak shape of these substances. All our attempts to obtain a reproducible deactivation procedure for nickel and stainless-steel tubings have met with little success. We found that the passivation steps recommended in the literature[9,32,34] for metal surfaces cause more harm than good in many cases. Furthermore, the electrolytic metal exchange that may occur between the tubing material and the metal chelate in the stationary phase when passivation is incomplete[6], poses a severe restriction to the use of metal capillary tubings in complexation GC.

Glass capillaries

In contrast to metal surfaces, glass surfaces show little of any activity towards metal chelates, and they can easily be adapted to the requirements of the stationary phase as well as to those of the solutes. For conventional GC stationary phases, methods of column pre-treatment are well established[35–38]. However, these procedures cannot be simply adopted for glass capillaries because of the inherent properties of the metal complex-containing stationary liquid. In complexation GC the chemical interaction of the coordinately unsaturated metal chelate with polar groups of the stationary liquid and/or the modified glass surface must be nil, or at least lower than that with the solute, in order to achieve a retention increase leading to selective separation through coordination. Liquids that are accordingly suitable as solvents for bis-β-ketoenolates of manganese(II), cobalt(II), nickel(II) and copper(II) are hydrocarbons of low volatility, *e.g.* squalane, Apiezon and Apolan, chlorofluorocarbons, *e.g.* Fluorolube oils, and non-polar or medium-polar silicon oils, *e.g.* OV-101, OV-1, OV-17 and SE-30. Polyether and polyglycol phases are inappropriate because they block the free coordination sites of the metal chelates. With metal capillary columns, squalane has generally been applied as the stationary liquid, and solutions of the metal chelates in silicon fluids showed only a poor wettability for nickel and stainless-steel surfaces. In contrast, with glass capillaries, squalane solutions formed droplets even on modified surfaces. Therefore, solutions of the metal chelates in silicon phases have been applied throughout the present studies.

For efficient deactivation of the glass surface it is necessary that all free silanol groups are completely removed. A protective functionalization of silanol groups can be carried out by the silylation procedure advocated by Grob *et al.*[39,40]. Yet in case of metal chelate-containing silicon fluids, droplets are still visible after coating. To enhance the wettability we tried to modify the surface with reagents that seemed to be more compatible with the stationary phases used. The method was to seal the column walls with insoluble organic polymers, and deactivation with Carbowax 20M gave the most promising results. The thermal polymerization of a dynamically coated Carbowax 20M film[41] was as successful as the procedure recommended by Franken *et al.*[31], which utilizes the volatiles emerging from a packed pre-column. Furthermore, glass capillaries for use in complexation GC can be efficiently deactivated with

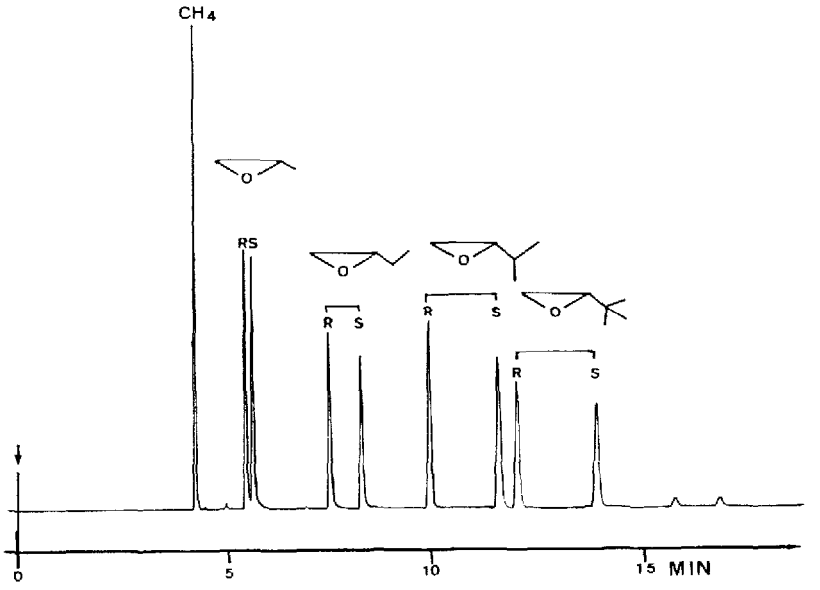


Fig. 1. Enantiomer separation of monoalkyl-substituted oxiranes on a 42 m × 0.25 mm I.D. Duran glass capillary coated with (2a) in OV-101 (0.06 *m*). Oven temperature, 40°C; carrier gas, nitrogen (0.7 bar).

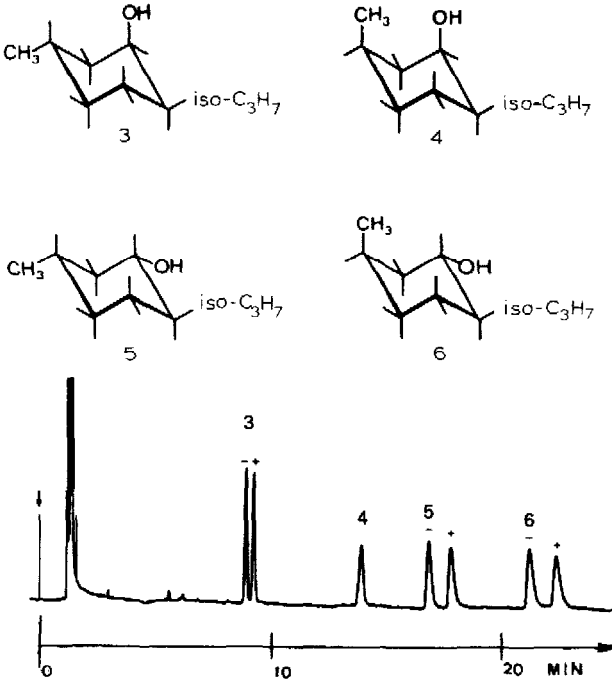


Fig. 2. Diastereomer and enantiomer (except for 4) separation of 3-menthanols on a 28 m × 0.25 mm I.D. Duran glass capillary coated with (2d) in OV-101 (0.08 *m*). Oven temperature, 116°C; carrier gas, nitrogen (1.0 bar). Compound: 3 = *neo*-menthol; 4 = *iso-neo*-menthol; 5 = menthol; 6 = *iso*-menthol[25].

Fig. 3. Enantiomer separation of the pheromone sulcatol on a 42 m × 0.3 mm I.D. Duran glass capillary coated with (2a) in OV-101 (0.08 *m*). Oven temperature, 60°C; carrier gas, nitrogen (0.5 bar).

silicons. We obtained wettable surfaces by thermal polymerization of the methylsilicon OV-101[42] or the methylphenylsilicon oligomer SY-231[43]. These capillaries can be coated with solutions of metal-β-ketoenolates in silicon phases. The average coating efficiency is greater than 80% ($k' = 10$, *n*-dodecane) and the adsorptivity of the column wall towards polar compounds is fairly low.

The chromatograms shown in the figures indicate the high efficiency of chemically modified glass open tubular columns for the quantitative separation of enantiomers and diasteromers by complexation GC. Fig. 1 shows the resolution of four racemic monoalkyl substituted oxiranes in 15 min, compared with 70 min with a metal capillary of slightly inferior performance[13]; the glass capillary column had a coating efficiency[22] of 95% ($k' = 10$, *n*-dodecane).

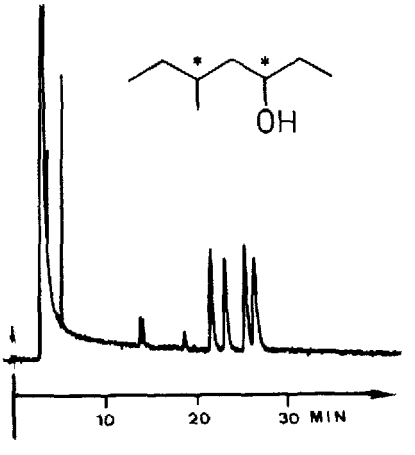


Fig. 4. Separation of the two enantiomeric pairs of 5-methylheptan-3-ol on a 37 m × 0.25 mm I.D. Duran glass capillary coated with (2d) in OV-101 (0.08 *m*). Oven temperature, 88°C; carrier gas, nitrogen (1.0 bar).

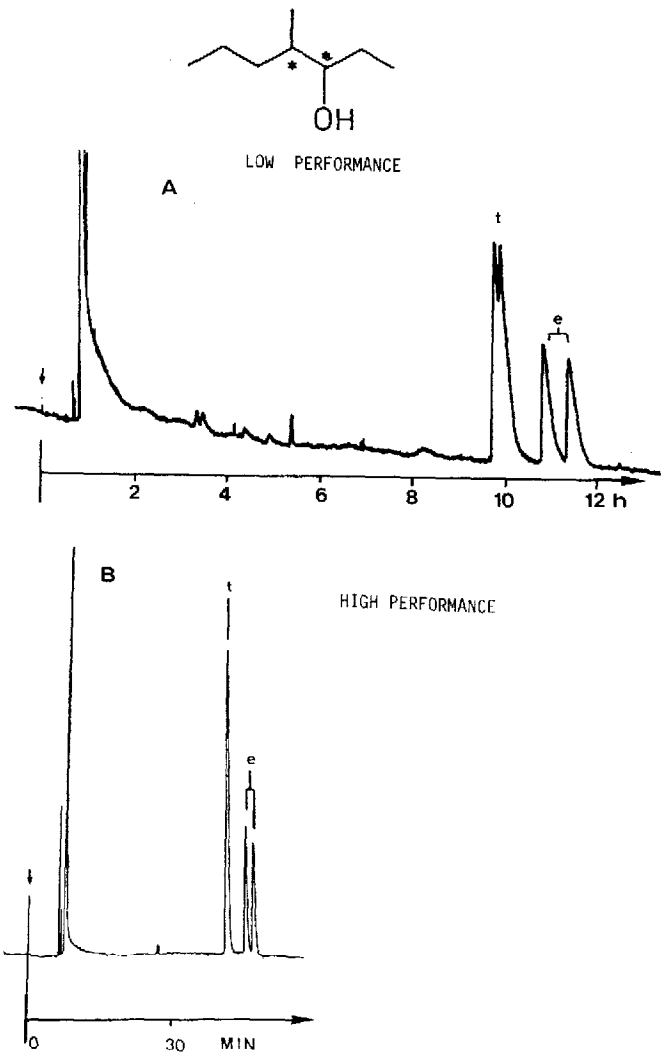


Fig. 5. Separation of the configurational isomers of the pheromone 4-methylheptan-3-ol (t = *threo*, e = *erythro* diastereomers). (A) A 160 m × 0.3 mm I.D. stainless-steel capillary coated with (2a) in squalane (0.13 *m*). Oven temperature, 45°C; carrier gas, nitrogen (0.5 bar). (B) A 42 m × 0.25 mm I.D. Duran glass capillary coated with (2d) in OV-101 (0.6 *m*). Oven temperature, 65°C; carrier gas, nitrogen (0.5 bar).

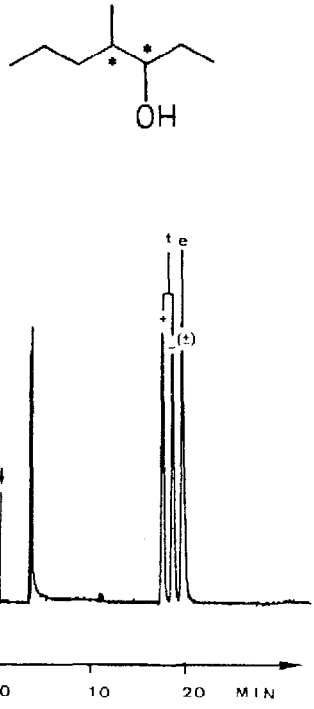
Selective separations of underivatized alcohols and ketones by complexation GC mostly benefit from the use of glass and fused-silica column technology. Fig. 2 shows the quantitative separation of the four diastereomeric 3-menthanols, *i.e.*, *neo*-menthol (3), *iso-neo*-menthol (4), menthol (5) and *iso*-menthol (6), as well as the concomitant baseline resolution into the antipodes, except for (4)[25]. For practical purposes the present approach to selective separation of configurational isomers of carbinols is superior to that previously described for alcohols[44], because it does not necessitate derivatization steps.

Biologically active chiral secondary alcohols are widespread in nature. Fig. 3

shows the quantitative separation of underivatized sulcatol (6-methyl-5-hepten-2-ol), the population aggregation pheromone of *Gnathotrichus sulcatus*[45]. Fig. 4 shows the resolution of all the configuration isomers of 5-methyl-heptan-3-ol, possessing two chiral centres and hence occurring as two (*erythro* and *threo*) pairs of enantiomers. Fig. 5 shows the separation of the configuration isomers of 4-methylheptan-3-ol, a pheromone constituent of the elm bark beetle *Scolytus multistriatus*[46], on a 42-m Duran glass capillary compared with the analysis on a 160-m stainless-steel capillary. On the steel capillary the broad and tailed peaks (asymmetry factor greater than 1.7) are eluted within 12 h, whereas the separation on the glass open tubular column takes only 0.7 h.

The impact of the nature of the resolving metal chelate on separation is indicated by a comparison of Figs. 5B and 6. When the manganese chelate (2a) is replaced by the nickel chelate (2d) the resolution of the *threo* isomer into the antipodes occurs. The chromatogram in Fig. 7 illustrates the enantiomer separation of an underivatized aliphatic ketone, *i.e.* the pheromone 4-methylheptan-3-one.

Shorter columns imply shorter retention times, and consequently the solute peaks are narrower and higher, resulting in a greater overall sensitivity of detection. The lower bleeding and the lower heat capacity of the glass open tubular columns compared with metal capillaries allow the use of on-column concentration techniques (*e.g.* splitless or on-column injection). This is very important for the analysis of trace components in complex mixtures of biological origin. We used the splitless injection technique and mass spectrometric "selected ion monitoring" as a substance-specific



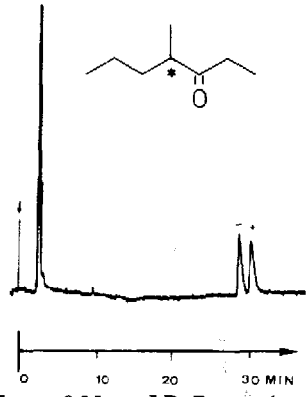


Fig. 6. Separation of *threo*-4-methylheptan-3-ol into enantiomers on a 37 m × 0.25 mm I.D. Duran glass capillary coated with (2d) in OV-101 (0.08 *m*). Oven temperature, 88°C; carrier gas, nitrogen (1.0 bar).

Fig. 7. Enantiomer separation of 4-methylheptan-3-one on a 37 m × 0.25 mm I.D. Duran glass capillary coated with (2d) in OV-101 (0.08 *m*). Oven temperature, 66°C; carrier gas, nitrogen (0.6 bar).

detector to determine the enantiomeric compositions of *exo*- and *endo*-brevicomin in *n*-pentane extracts of *Dendroctonus ponderosae* and *Dryocetes confusus*, which are major bark-beetle pests in the northern hemisphere[28]. Likewise, the enantiomeric excess of lineatin in the boring dust of three *Trypodendron* beetles was measured[27]. The prerequisite for the monitoring of enantiomeric compositions of trace amounts of natural substances is the highly efficient and sensitive baseline resolution of the underivatized solute. For acetals, complexation GC is the only method available at present[16–19]. The quantitative separation (Fig. 8) of the optical isomers of frontalin, the aggregation pheromone of the Southern pine beetle *Dendroctonus frontalis*, serves as an instructive example[28].

Fused-silica capillaries

Since the first report of the use of fused-silica capillary columns in GC by Dandeneau and Zerenner[47], these columns have attained increasing importance because of the high inertness of the surface and the flexibility of the tubing. Yet the untreated quartz surface is barely wettable by most stationary liquids. The high drawing temperature of quartz (1800°C) produces a dehydroxylated surface[48]. This means that the surface mainly consists of Si–O–Si bridges, which can be readily hydrolysed by polar compounds. Therefore special treatment is necessary prior to coating in complexation GC. In order to prepare a hydroxylated surface, which is essential for most deactivation procedures, the capillary is treated with mineral acid (hydrothermal treatment)[30]. For the use of fused-silica capillaries in complexation GC we found

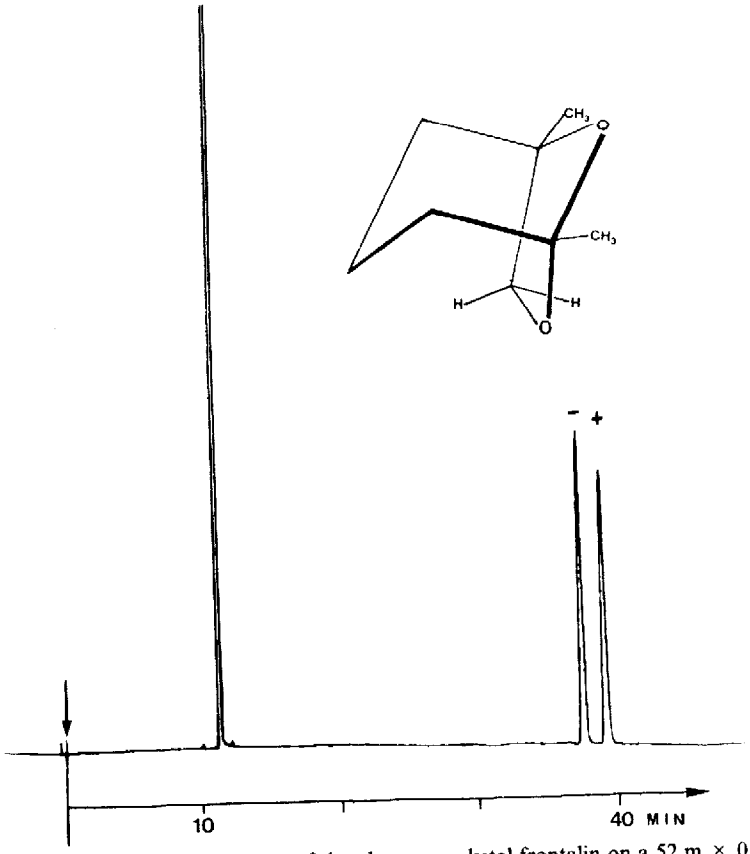
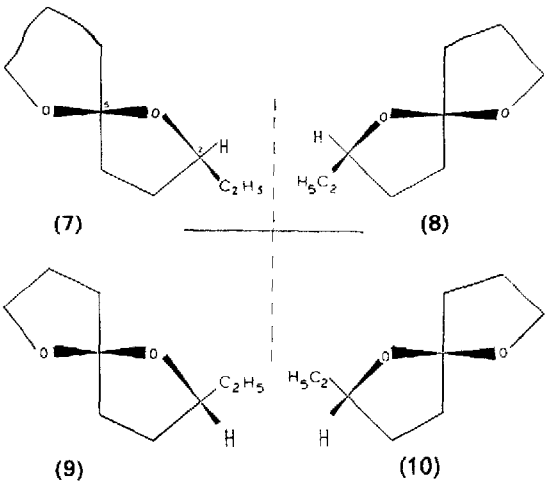


Fig. 8. Enantiomer separation of the pheromone ketal frontalin on a 52 m × 0.25 mm I.D. Duran glass capillary coated with (2a) in OV-101 (0.075 *m*). Oven temperature, 62°C; carrier gas, helium (0.8 bar).



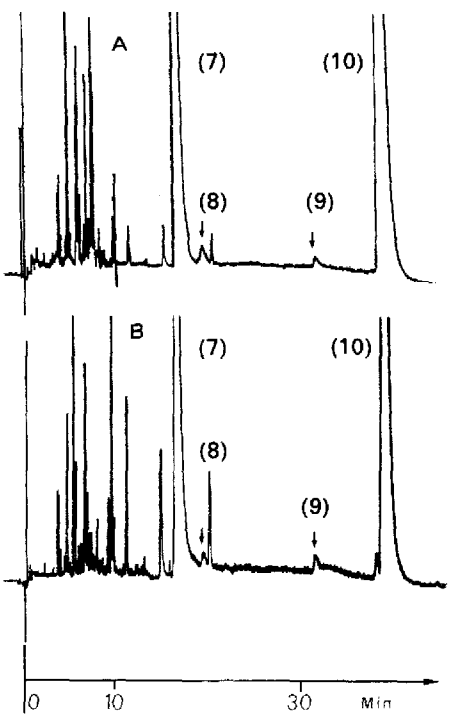


Fig. 9. Mass fragmentogram (m/e = 127) of the defatted *n*-pentane extracts of *Pityogenes chalcographus* and *P. quadridens*. GC conditions: 25 m × 0.25 mm I.D. fused-silica capillary coated with a solution of (2d) in OV-101 (0.1 *m*). Oven temperature, 75°C; carrier gas, helium (0.8 bar); 1.5-μl extract; splitless injection. MS conditions: Varian MAT 112S; ionizing voltage, 70 eV, interface temperature, 120°C; ion source temperature, 200°C; resolution, 1000. (A) Extract of *P. chalcographus*: (2*S*,5*S*)-isomer (7), enantiomeric composition, 98.8 ± 1%; (2*S*,5*R*)-isomer (10), enantiomeric composition, 99.2 ± 1%. (B) Extract of *P. quadridens*: (2*S*,5*S*)-isomer (7), enantiomeric composition, 98.7 ± 1%; (2*S*,5*R*)-isomer (10), enantiomeric composition, 99.0 ± 1%.

that the column can be deactivated by Carbowax 20M in essentially the same manner as described for glass capillaries. After coating with solutions of metal chelates in OV-101 highly efficient columns are obtained. These capillaries are especially suitable for use in GC mass spectrometry coupling techniques because of the low bleeding and their flexibility. Thus, a fused-silica capillary column can be installed near the ion-source of the mass spectrometer without the aid of an auxiliary coupling capillary tube. Fig. 9 shows the application of a 25-m fused-silica open tubular column coated with nickel(II) bis(3-heptafluorobutanoyl-1*R*-camphorate) (2d) in OV-101 to the direct configurational analysis of 2-ethyl-1.6-dioxaspiro(4.4)nonane (Chalcogran)[49], the major pheromone component of *Pityogenes chalcographus* and *P. quadridens*. The determination was done directly out of the defatted but otherwise unpurified *n*-pentane extract of the beetles. Digital peak integration revealed only slight differences in the configurational composition of the spiro-ketal in both species.

CONCLUSIONS

Glass and fused-silica open tubular columns have several advantages over metal capillaries in complexation GC for the selective and direct separation of structural, configurational and optical isomers: (i) shorter columns; (ii) reduced retention times; (iii) higher sensitivity; (iv) higher thermal stability; (v) lower bleeding; (vi) lower costs.

The lower bleeding and the lower heat capacity of the columns mean that on-column concentration techniques, *e.g.* splitless injection, are applicable. This allows the determination of the configurational compositions of various resolvable solutes with a minimal amount of substance (*ca.* 1 ng) and an accuracy of better than ± 1%. Various applications of the columns in the fields of diastereo- and enantioselective chemical synthesis, natural product characterization and resolution of multi-component mixtures can be envisioned.

It is important to note that the glass and fused-silica columns employed in this work possessed extended lifetimes, *i.e.* no change of efficiency and resolvability was observed within two years.

NOTE ADDED IN PROOF

The commercialization of Capillary Columns for Complexation Chromatography (CC & CC) is forthcoming (consult authors for information).

ACKNOWLEDGMENTS

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