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HIGHLY SENSITIVE DIASTEREOISOMERIC ANALYSIS OF SECONDARY ALCOHOLS AND SHORT-CHAIN 1,2-DIACYLGLYCEROLS BY CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

PETER MICHELSEN*

Division of Organic Chemistry 1, Chemical Center, University of Lund, P.O. Box 124, S-221 00 Lund (Sweden)

and

GÖRAN ODHAM

Laboratory of Ecological Chemistry, University of Lund, Ecology Building, Helgonavägen 5, S-223 62 Lund (Sweden)

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SUMMARY

    Diastereomeric separation of short-chain 1,2-diacylglycerols has been carried out by high-resolution gas chromatography. A new versatile chiral reagent, R-(+)-2-phenylselenopropionic acid, was investigated and compared with α-methoxy-α-trifluoromethylphenylacetic acid and α-methoxy-α-methylpentafluorophenylacetic acid as diastereomeric esters of 2-octanol. By use of the mass fragmentographic technique, it was shown that diastereomeric analysis can be performed in the femtomole range.

INTRODUCTION

    To elucidate the action of the various lipases and phosphatidases on glycerides, as well as the general stereochemistry of these important lipids, there has been considerable interest in developing reliable and sensitive methods for enantiomeric analyses of degradation products of the diacylglycerol type. The methods used involve studies with model compounds[1] or alkyl analogues[2] labeled with radioactive or stable isotopes. For determination of the stereochemistry of the glycerols, the techniques of optical rotary dispersion and circular dichroism have been most commonly employed[3,4]. However, the large amount of sample needed in such optical studies, typically in the milligram range, has restricted the number of investigations carried out. Thus, there is a great need for more sensitive methods for enantiomeric determinations of diacyl-*sn*-glycerols containing fatty acid moieties of variable size.

    A method often used for the determination of the enantiomeric purity of simple chiral alcohols is based on their conversion into diastereoisomeric mixtures with suitable optically active reagents, followed by analyses with chromatographic or NMR spectroscopic techniques. A reagent commonly employed for such analyses is α-methoxy-α-trifluoromethylphenylacetic acid (Moscher's MTPA reagent), which is

commercially available in both enantiomeric forms[5]. In addition to their usefulness in ^{19}F NMR spectroscopy, diastereoisomers formed with Moscher's reagent are well suited to gas chromatographic (GC) analysis because of their relatively high volatility and the availability of different detector systems.

The present study concerns investigations of the possibility of using capillary GC techniques for enantiomeric analysis of short-chain 1,2-diacylglycerols by separation of diastereoisomers formed by derivatization with Moscher's MTPA reagent.

Parallel to these studies, other reagents for the formation of diastereoisomeric derivatives were investigated, including α-methyl-α-methoxypentafluorophenylacetic acid and 2-phenylselenopropionic acid[6]. The latter acid is also useful in ^{77}Se NMR spectroscopy.

EXPERIMENTAL

Reagents

The syntheses of R,S- and R-(+)-2-phenylselenopropionic acid and its acid chloride are described in ref. 6. R,S-α-Methoxy-α-trifluoromethylphenylacetic acid (MTPA), commercial S-(−)-α-methoxy-α-trifluoromethylphenylacetic acid (Jansson), and their corresponding acid chlorides, were prepared according to ref. 5. Synthetic α-methyl-α-methoxypentafluorophenylacetic acid and the corresponding acid chloride were prepared according to ref. 7. All solvents used were distilled in the laboratory prior to use. R-(+)-2-Octanol was purchased from Fluka (Buchs, Switzerland).

Synthesis

Various diastereomeric mixtures of R-(+)-2-octanol were prepared according to ref. 6. The synthetic routes to various 1,2-diacyl-3-α-methoxy-α-trifluoromethylphenylacetic acid glycerols are shown in Scheme 1.
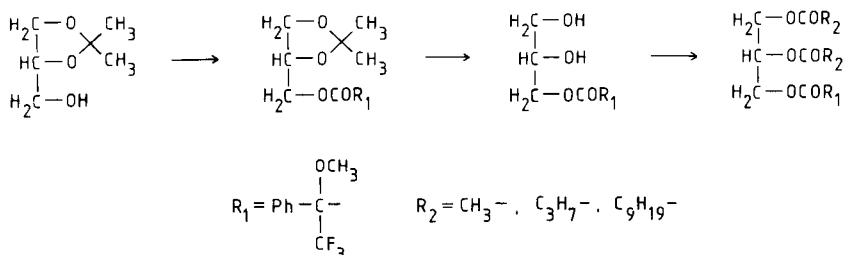
The synthesis of rac-1,2-isopropylidene-3-α-methoxy-α-trifluoromethylphenylacetic acid was analogous to that described in ref. 4 in 20% yield. The isopropylidene protecting group was removed with 45% acetic acid at 40°C for 3 h, according to ref. 8. From this compound various 1,2-diacyl derivatives were prepared: diethanoyl and dibutanoyl derivatives were acylated with the corresponding acid anhydride in toluene–pyridine and worked up according to ref. 4. The didecanoyl derivative was prepared using the corresponding acid chloride and worked up analogously to the description in ref. 4. This procedure was used because it was not possible to obtain

the desired diastereomeric derivatives directly by acylation of 1,2-diacylglycerols with α-methoxy-α-trifluoromethylphenylacetic acid chloride. This is probably due to the low reactivity of the reagent.

Gas chromatography–mass spectrometry

A fused-silica column (25 m × 0.2 mm I.D.) deactivated with octamethylcyclotetrasiloxane and statically coated with SE-54 in the laboratory, was used for the separation of diastereoisomers.

In the flame ionization detection (FID) experiments, a Varian Model 3700 instrument, equipped with an all-glass split/splitless injection system, was used. The chromatographic conditions were: injector temperature, 280°C; detector temperature, 300°C. Oven parameters for each diastereoisomer are given in the figure captions. The nitrogen carrier gas flow-rate was 20 cm/sec and the make-up gas flow-rate was 30 ml/min. In split injections, the split ratio was *ca.* 1:100, and in splitless injections the injector was vented 45 sec after the injection.

The mass spectrometer was a Ribermag R10-10c quadrupole GC mass spectral data acquisition system equipped with a Carlo Erba Model 4160 GC. Helium was used as carrier gas at a flow-rate of 40 cm/sec. Other GC conditions used were as described for the FID studies. The chemical ionization reagent gas (ammonia) was ionized at 94 eV and had a purity greater than 99.95%. The ion source temperature was 180°C and the pressure 0.1 Torr.

RESULTS AND DISCUSSION

One aim of this work was to study at least two derivatization reagents, and compare them with the previously described 2-phenylselenopropionic acid[6].

The GC separations of diastereoisomeric esters of *R*-2-octanol and *R,S*-2-phenylselenopropionic acid. *RS*-α-methyl-α-methoxypentafluorophenyl acetic acid (MMPA) and *RS*-α-methoxy-α-trifluoromethylphenyl acetic acid (MTPA), are shown in Fig. 1. The calculated corresponding resolution values (*R*) were 1.00, 1.03 and 1.47, respectively. The diastereoisomers with shorter retention times are assigned the *S*-configuration of the acids and those with longer retention times the *R*-config-
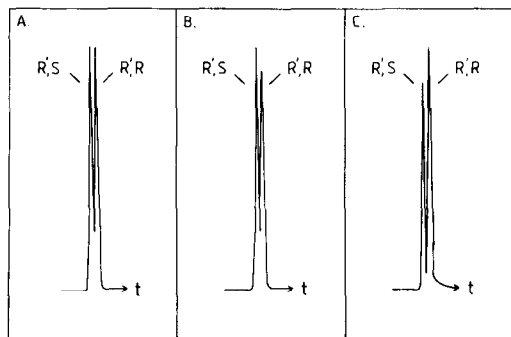

Fig. 1. Diastereomeric GC separations of *R*-(+)-2-octanol and: (A) *R,S*-2-phenylselenopropionic acid; (B) *R,S*-α-methoxy(pentafluorophenyl)acetic acid; (C) *R,S*-α-methoxy-α-trifluorophenylacetic acid. Column temperature, 200°C.
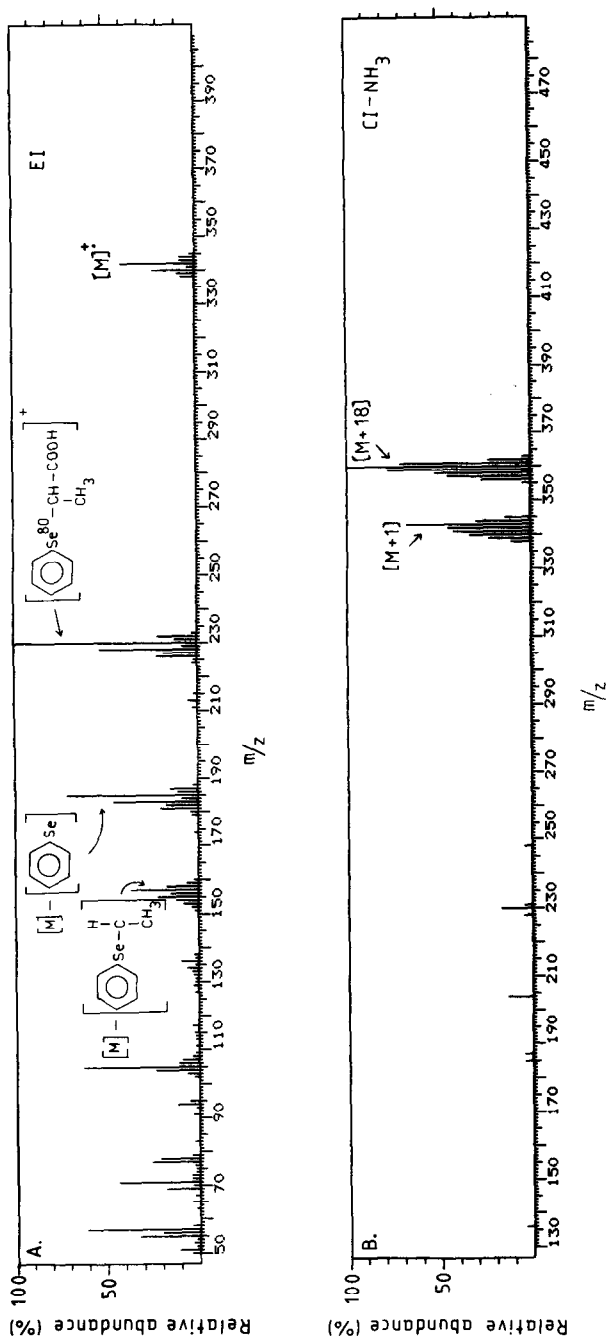
Fig. 2. Mass spectra of the 2-phenylselenopropionic ester of 2-octanol; (A) electron impact and (B) chemical ionization with ammonia.

uration. This was established for A[3] and C[4] with known pure enantiomeric samples, whereas the assignment for B is only tentative[1,2].

Another aim of these studies was to achieve very high sensitivity and selectivity in the analyses. Mass spectrometry (MS) adapted for selected-ion monitoring (SIM) constitutes a detection technique with such capabilities, and the diastereoisomeric pair in Fig. 1A was therefore subjected to such MS studies.

The full mass spectra, electron impact (EI) ionization, and chemical ionization (CI), of R-2-octanol R-2-phenylselenopropionic acid ester, with ammonia as reagent gas, are shown in Fig. 2. Both spectra indicate large abundances of molecular ions (EI) at m/z 342 (most common isotope) or adduct ions (CI) at m/z 360 (M + 18). Since SIM in CI usually provides a somewhat higher sensitivity for esters than EI[9], it was chosen for the mass fragmentographic study.

In Fig. 3, a typical fragmentogram obtained from 1.5 pmol of the diastereo-isomeric mixture is reproduced. Accepting a signal-to-noise ratio of 2, we estimated the detection limit to be ca. 300 fmol.
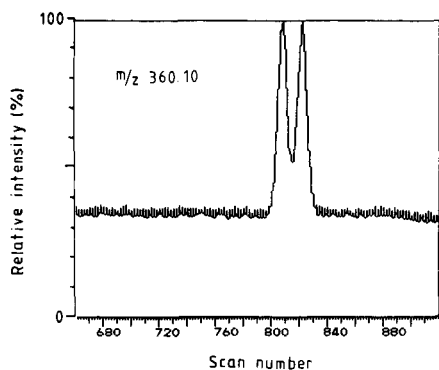

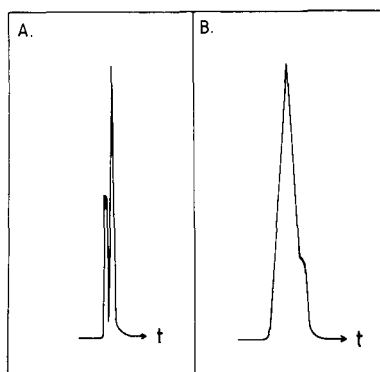Fig. 3. Fragmentogram of the 2-phenylselenopropionic esters of 2-octanol (CI with ammonia).

Fig. 4. Diastereomeric GC separations of MTPA esters of (A) rac-1,2-diethanoylglycerol and (B) rac-1,2-didecanoylglycerol. Column temperature, 190°C.
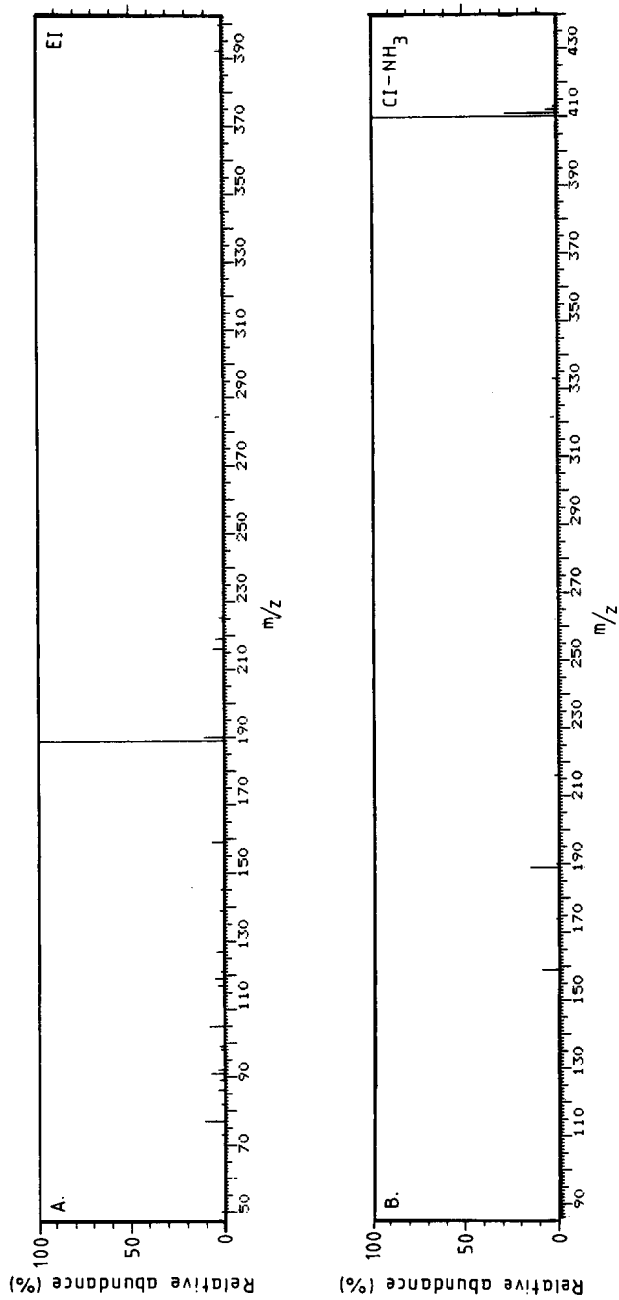
Fig. 5. Mass spectra of 1,2-diethanoylglycerol ester of MTPA; (A) electron impact and (B) chemical ionization with ammonia.

It may be concluded that 2-phenylselenopropionic acid is a versatile reagent for testing the enantiomeric purity of chiral alcohols. If unlimited amounts of sample are available, ^{77}Se NMR spectroscopy may be used to advantage because the technique allows very simple interpretation of the measurements[6]. However, with very small samples, the picomole or femtomole ranges, chromatographic techniques with FID or MS detection must replace the spectroscopic techniques.

We also investigated the possibility of using capillary GC for the separation of diastereomeric diacylglycerols obtained by acylation with a chiral acid. For the elucidation of diastereomeric GC separations of short-chain 1,2-diacylglycerols, MTPA was chosen as derivatization reagent. MTPA is a particularly attractive acylating agent because both enantiomeric forms are commercially available with high enantiomeric purity.

The diastereomeric capillary GC separations of 1,2-diethanoyl-, 1,2-dibutyryl and 1,2-didecanolglycerol esters of α-methoxy-α-trifluoromethylphenylacetic acid were investigated. The diethanoyl diastereomers exhibit nearly baseline separation with a resolution value (R) of 1.02 (Fig. 4A), whereas only slight separation of the didecanoyl derivative was observed (R ca. 0.3; Fig. 4B). The proportions of the two diastereoisomers varied considerably with the experimental conditions. Acylation with the chiral trifluoromethyl-substituted acid obviously proceeds in a strongly stereoselective manner and, as a consequence, requires long reaction times. However, it is not possible to increase the reaction time above 12 h because of acyl migration problems.

Full mass spectra, EI and CI (ammonia), of the 1,2-diethanoylglycerol ester of MTPA were recorded (Fig. 5). Both spectra show abundant ions of m/z 189, whereas only CI shows an adduct ion at m/z 410 (M + 18). Choosing this ion, it was possible to make the analysis in the 0.1 pmol range (CI), using SIM (Fig. 6). The detection limit in this case was estimated to be in the same range as previously described, assuming a signal-to-noise ratio of 2.

However, since long-chain homologues of 1,2-diacyl diastereomers were not satisfactorily resolved on the GC capillary system, there was no further need to continue the investigation with other chiral acid reagents. This is because long-chain 1,2-diacylglycerols are of greatest interest, owing to their dominance in naturally occurring lipids. To analyse medium- or long-chain 1,2-diacylglycerols, other methods including liquid chromatographic techniques must be used[10].
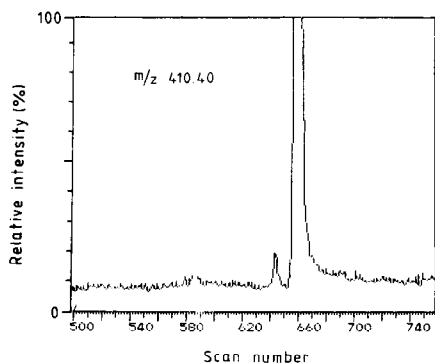

Fig. 6. Fragmentogram of 1,2-diethanoylglycerol ester of MTPA in the 0.1-pmol range (CI with ammonia).

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