Journal of Chromatography, 351 (1986) 363–365
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 230

Note

Capillary gas–liquid or thin-layer chromatographic resolution of 2-hydroxy-fatty acid enantiomers

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(First received June 25th, 1985; revised manuscript received September 30th, 1985)

There have been several reports on the separation of enantiomers; some have described direct separation[1], although the most common method has been the use of chiral reagants producing diastereomeric derivatives or chromatographic chiral phases[2–7]. Methods using oxidative ozonolysis followed by chemical modification have enabled determination of the absolute configuration of hydroperoxides of unsaturated fatty acids[8,9].

We report here a technique for the direct separation of the diastereomeric D and L forms of hydroxylated fatty acids whose hydroxyl and carboxylic groups are close together. This separtion can be carried out either by capillary gas–liquid chromatography (GLC) or by thin-layer chromatography (TLC) using freshly prepared silicone-bonded plates.

EXPERIMENTAL

Chemicals

The thin-layer silica-coated plates were from Merck. Phenylmethylvinylchlorosilane, (−)-α-methoxy-α-trifluoromethylphenylacetic acid and 2-DL-hydroxypalmitic acid were from Fluka (Switzerland); 2-DL-hydroxystearic acid was from Larodan (Sweden).

Derivatization procedures

α-Methoxy-α-trifluoromethylphenylacetic acid (MTPA) was converted to the active acid chloride derivative using the method described by Dale[10]. Briefly, MTPA is refluxed for 50 h in the presence of a mixture of thionyl chloride and sodium chloride. After removal of excess thionyl chloride, the acid chloride is diluted 1:100 in pyridine. A 200-μl volume of a solution of MTPA acid chloride is added to 200 μg of hydroxylated fatty acid. The mixture is left for 12 h at 60°C and the solvent is then evaporated.

0021-9673/86/$03.50

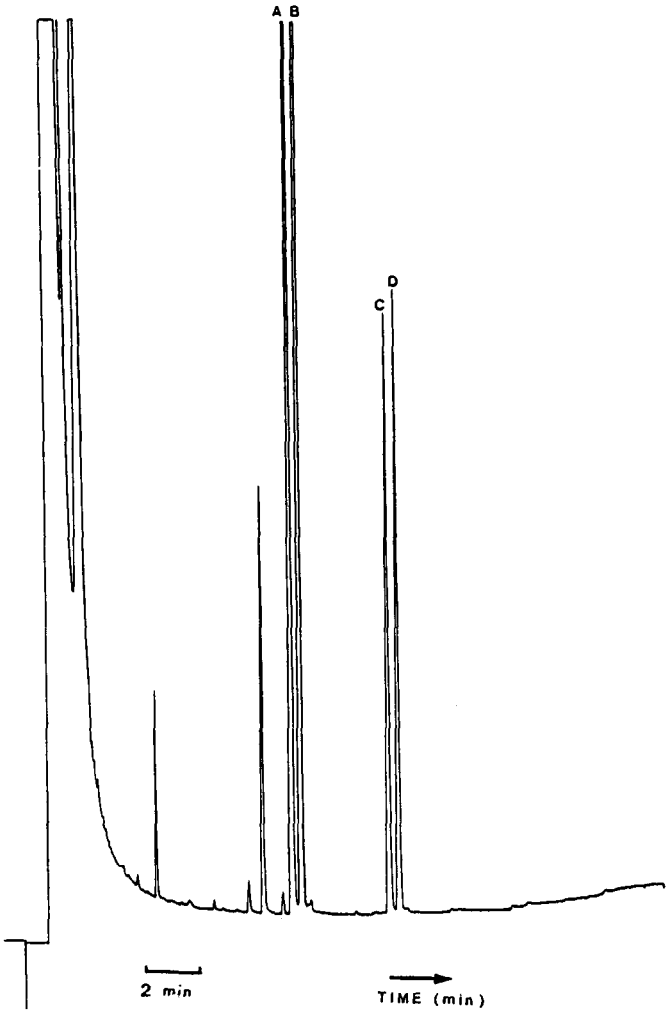

Fig. 1. Capillary gas–liquid chromatogram of diastereomeric derivatives (with MTPA chloride) of methyl-2-hydroxypalmitate (A, B) and methyl-2-hydroxystearate (C, D). Column (25 m × 0.32 mm I.D.) coated with OV-1; carrier gas helium; column temperature programmed from 180 to 240°C at 2°C/min.

Chromatography

Thin-layer silica-coated plates are prepared by an *in situ* chemical reaction using phenylmethylvinylchlorosilane, as described in a previous publication[11]. GLC was carried out in high-efficiency fused-silica columns (25 m × 0.32 mm I.D.) specially made in the laboratory[12]. Their chromatographic characteristics are as follows: stationary phase thickness 0.20 μm, theoretical plates >3000/m, efficiency $>80\%$, stationary phase OV-1.

The diastereomeric derivatives were treated with diazomethane and analysed by capillary GLC–mass spectrometry (MS) using an LKB 2091 0-61 apparatus with a temperature programme from 180 to 240°C at 2°C/min.

RESULTS

Fig. 1 shows the separations of methyl-2-DL-hydroxy palmitate into two equal peaks A and B, and methyl-2-DL-hydroxystearate into two equal peaks C and D. Peaks A and B had identical mass spectra, as did peaks C and D. It can be seen that the D and L forms of the hydroxylated fatty acids were separated. The order of elution of diastereoisomers was studied as far as pure enantiomers were available.

In the case of malic acid, the L enantiomer has the longer retention time, and we thus assume that for 2-hydroxy-fatty acid enantiomers, the D form is the first compound to be eluted.

Unmodified and phenylmethylvinylchlorosilane-coated plates are spotted with two 2-μl aliquots of a solution of diastereomeric derivatives of 2-DL-hydroxypalmitic acid, and then developed with heptane–methyl formate–diethyl ether–acetic acid (50:40:10:2). The distance travelled by the solvent is 8 cm. The plates are dried at 110°C and sprayed with molybdic acid–ethanol (2:98). Only one spot with as R_F value of 0.12 is observed on the untreated plates. On the treated plates, two spots are observed with R_F values of 0.30 and 0.34. In order to check that the D and L isomers were separated, a further migration was carried out. The spots were scraped off and eluted. They were then methylated with diazomethane, and analysed by capillary GLC–MS. The two peaks had identical mass spectra.

Using (−)-α-methoxy-α-trifluoro-5-methylphenylacetic acid, the D and L forms of monohydroxylated fatty acids with 16–18 carbon atoms can be separated, provided that the hydroxyl and carboxylic groups are close together.

We are carrying out investigations on the separation of hydroxylated fatty acids where the hydroxyl group is in the middle of the chain. This method shows promise since the molecules do not have to be split into various fragments before preparation of the chiral derivatives.

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