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Note

Gas–liquid chromatographic separation of geometric isomers of unsaturated fatty acid methyl esters using a glass capillary column

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The *cis*, *trans* and certain positional isomers of unsaturated fatty acids are of potential biochemical interest[1]. Many methods for the separation of geometric and positional isomers of mono-, di- and triethylene unsaturated fatty acid methyl esters have been reported[2–13]. The advent of highly polar polysiloxane stationary phases such as SP-2340, OV-275, Silar-10C and Silar-9CP have made it possible to separate geometric and positional isomers of fatty acid methyl esters. These cyanopropylsiloxane stationary phases have high polarity and temperature stability. In this work the highly polar stationary phase SS-4, a cyanoethyl polysiloxane with good temperature stability was investigated as a glass capillary column coating for the separation of mono-, di- and triethylene fatty acid methyl esters. For linolenic acid, there are theoretically eight isomers, but as yet the eight isomers have not been separated[13]. In this study the gas chromatogram of linolenic acid obtained with a 60-m glass capillary column showed eight peaks. Identification of the isomers is discussed. The fatty acid composition of lung tissue phospholipids was also analysed using a 40-m glass capillary column coated with SS-4.

EXPERIMENTAL

Materials

All pure samples of *cis*-9-hexadecenoic acid (palmitoleic acid), *trans*-9-hexadecenoic acid (palmitelaidic acid), *cis*-9-octadeconoic acid (oleic acid), *trans*-9-octadecenoic acid (elaidic acid), *cis*-11-octadecenoic acid (vaccenic acid), *cis*-9,*cis*-12-octadecadienoic acid (linoleic acid), *trans*-9, *trans*-12-octadecadienoic acid (linoelaidic acid) and *cis*-9,*cis*-12,*cis*-15-octadecatrienoic acid (linolenic acid) were obtained commercially. They were of known structure and their purity was better than 99%. Mixtures containing the *cis*/*trans* isomers of octadecadienoic and octadecatrienoic acids were prepared by exposing pure linoleic acid and linolenic acid to 500 ppm of nitrogen dioxide in carbon tetrachloride[14,15] and/or according to the nitrous acid isomerization method[16,17]. The chromatograms for both methods were identical with respect to the number of peaks and retention times. A partial separation of *cis*-9, *trans*-12- and *trans*-9,*cis*-12-octadecadienoic acid was achieved by preparative thin-layer chromatography on silver nitrate-impregnated silica gel[18]. The *trans*-9,

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trans-12,*trans*-15-octadecatrienoic acids were also separated by this method. These fatty acids were methylated with diazomethane in diethyl ether[19].

Fatty acids in lung phospholipids

A female Wistar rat (8 weeks old, body weight 148 g) was killed by exsanguination. The whole lung was homogenized in 3.3 ml of methanol–chloroform–water (2:1:0.3) and the lipids were extracted according to the method of Folch *et al.*[20]. Total lipids were separated into neutral lipids and phospholipids by thin-layer chromatography. Before the sample was applied to the gas chromatographic column, methanolysis of the phospholipids was done with 0.5 *N* methanolic sodium hydroxide under nitrogen for 5 min at 100°C and further reaction was effected with 14% boron-trifluoride–methanol under nitrogen for 10 min at 100°C[21]. Then 8 ml of water were added to the solution and the methyl esters were extracted with *n*-hexane.

Gas–liquid chromatographic analysis

Gas chromatography was performed on a Shimadzu GC-5A gas chromatograph equipped with a flame-ionization detector (FID). SCOT-type glass capillary columns (60 m × 0.3 mm I.D. and sometimes 40 m × 0.3 mm I.D. and 30 m × 0.3 mm I.D.) were used. The columns were coated with cyanoethyl polysiloxane of about 50% substitution (SS-4), developed by Shinwa Kako (Kyoto, Japan). The oven temperature was 175°C or sometimes 190°C. The detector temperature was 250°C and the injector temperature was 250°C. The flow-rate of the carrier gas (99.999% nitrogen) was 0.71 ml/min. The splitting ratio was 88:1.

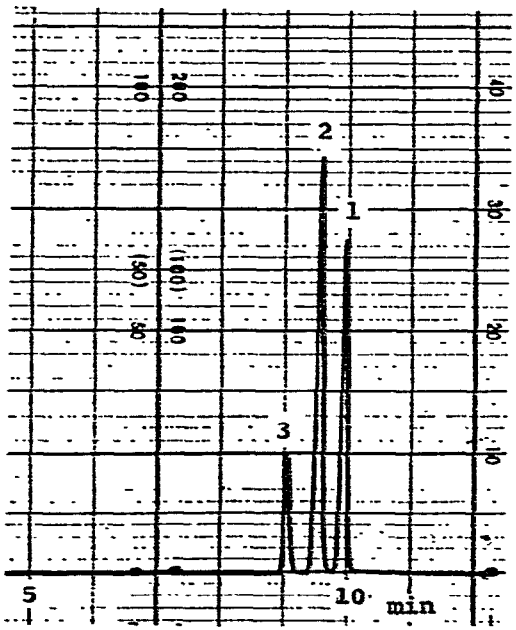

Fig. 1. Gas chromatogram of geometric isomers of oleic acid methyl ester on a glass capillary column (30 m × 0.3 mm I.D.) coated with SS-4. The column was operated at 190°C with nitrogen as the carrier gas at a flow-rate of 1.4 ml/min. Injector and detector temperatures, 250°C. Peaks: 1 = $C_{18:1}\Delta 9c$; 2 = $C_{18:1}\Delta 9t$; 3 = methyl stearate.

RESULTS AND DISCUSSION

The gas chromatogram for *cis*-9- and *trans*-9-octadecenoic acids is shown in Fig. 1; baseline separation was achieved. Baseline separation was also achieved with *cis*-9- and *trans*-9-hexadecenoic acids. Linoleic acid has four geometric isomers (*cis*-9,*cis*-12-,*trans*-9,*cis*-12-, *cis*-9,*trans*-12- and *trans*-9,*trans*-12-), and Fig. 2 shows the gas chromatogram of these four isomers produced by the reaction of linoleic acid with nitrogen dioxide. All-*cis*, all-*trans* and mono-*trans* isomers were completely separated. Among the mono-*trans* isomers, the *trans*-9,*cis*-12 isomer overlapped slightly with the *cis*-9,*trans*-12 isomer. On SP-2340 as the stationary phase, these mono-*trans* isomers showed baseline separation but the latter peak overlapped that of the all-*trans* isomer[13]. The proportions of these mono-*trans* isomers are almost equal. Therefore, Fig. 2 also reveals that nitrogen dioxide attacks the two double bonds of linoleic acid without preference.

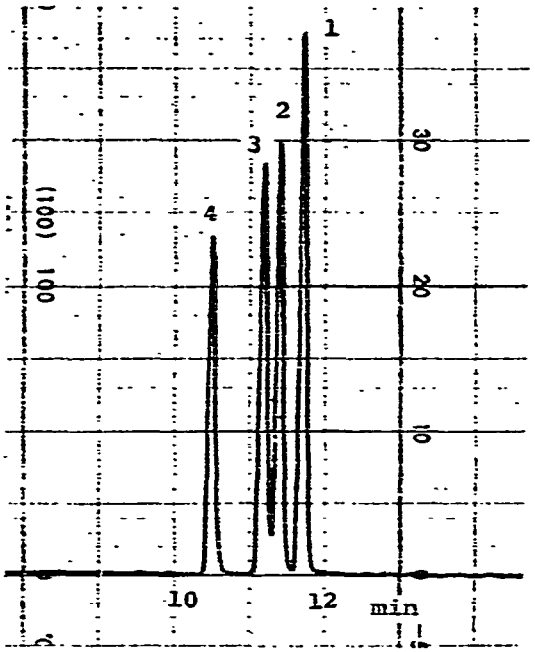

Fig. 2. Gas chromatogram of the four geometric isomers of the methyl esters of linoleic acid on a glass capillary column (30 m × 0.3 mm I.D.) coated with SS-4. Operating conditions as in Fig. 1. Peaks: 1 = $C_{18:2}\Delta 9c,12c$; 2 = $C_{18:2}\Delta 9t,12c$; 3 = $C_{18:2}\Delta 9c,12t$; 4 = $C_{18:2}\Delta 9t,12t$.

For linolenic acid there are, theoretically, eight possible isomers, but the separation of all eight has not previously been performed. Heckers *et al.*[13] obtained seven peaks by using a glass capillary column coated with SP-2340. We used a 60-m glass capillary column coated with SS-4 and obtained eight peaks, as shown in Fig. 3. However, 30- or 40-m using glass capillary columns, seven peaks were obtained, as reported by Heckers *et al.*[13]. Peaks 1 and 8 in Fig. 3 have been assigned to the all-*cis* and the all-*trans* isomer respectively. Six peaks (2, 3, 4, 5, 6 and 7), however, have not yet been assigned.

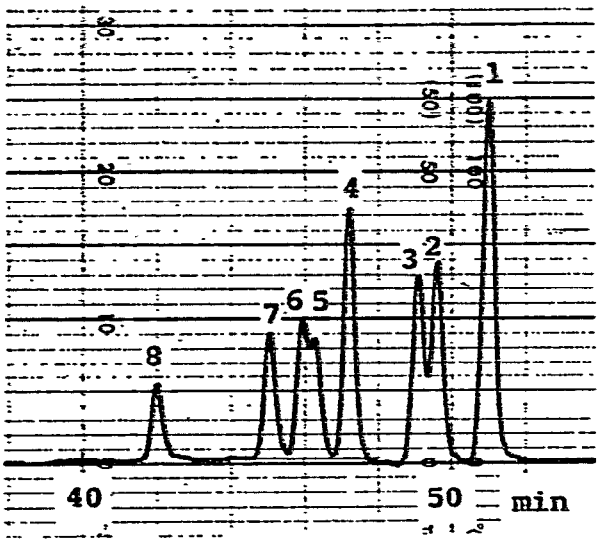

Fig. 3. Gas chromatogram of methylated reaction products of linolenic acid catalysed by nitrogen dioxide on a glass capillary column (60 m × 0.3 mm I.D.) coated with SS-4. The column was operated at 175°C with nitrogen as the carrier gas at a flow-rate of 0.7 ml/min. The splitting ratio was 88:1. Injector and detector temperatures, 250°C. Peaks: 1 = $C_{18:3}\Delta 9c,12c,15c$; 2–7 = $C_{18:3}$ (individual peaks were not assigned); 8 = $C_{18:3}\Delta 9t,12t,15t$.

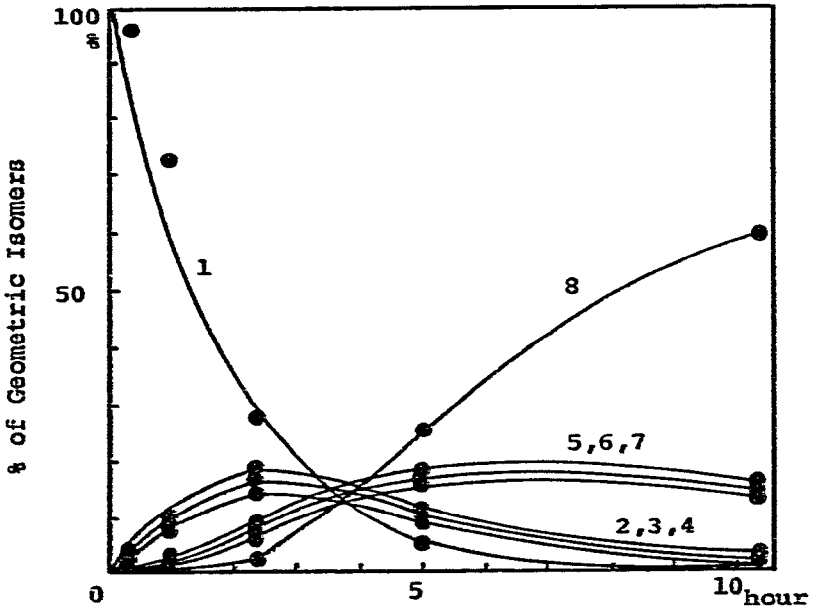

Fig. 4. *Cis–trans* isomerization of linolenic acid catalysed by nitrogen dioxide (477 ppm) in carbon tetrachloride. Nos. 1–8 refer to GC peaks shown in Fig. 3.

Fig. 4 illustrates the change in the proportions of the eight isomers for the reaction of linolenic acid with nitrogen dioxide. In this reaction isomerization of the all-*cis* isomer to the three mono-*trans* isomers occurred first. The three mono-*trans* isomers could then be converted into the three di-*trans* isomers. Next, the three di-*trans* isomers could be converted into the all-*trans* isomer. As shown in Fig. 4, peak 1 decreased continuously and the three peaks 2, 3 and 4 increased first. Therefore, peaks 2, 3 and 4 are assumed to correspond to the three mono-*trans* isomers. Then the three peaks 5, 6 and 7 increased and are assumed to be the three di-*trans* isomers. Peak 8, the all-*trans* isomer, increased continuously with time. For identification of the mono- and di-*trans* isomers, fractionation of the isomers, spectroscopic analysis and chemical techniques such as ozonization should be employed.

Fig. 5 shows the gas chromatogram of the methyl esters of the fatty acids associated with lung tissue phospholipids, obtained by using a 40-m SS-4 coated glass capillary column. A good separation of all the fatty acids of phospholipids was achieved. Positional isomers such as oleic acid and vaccenic acid methyl esters show baseline separation.

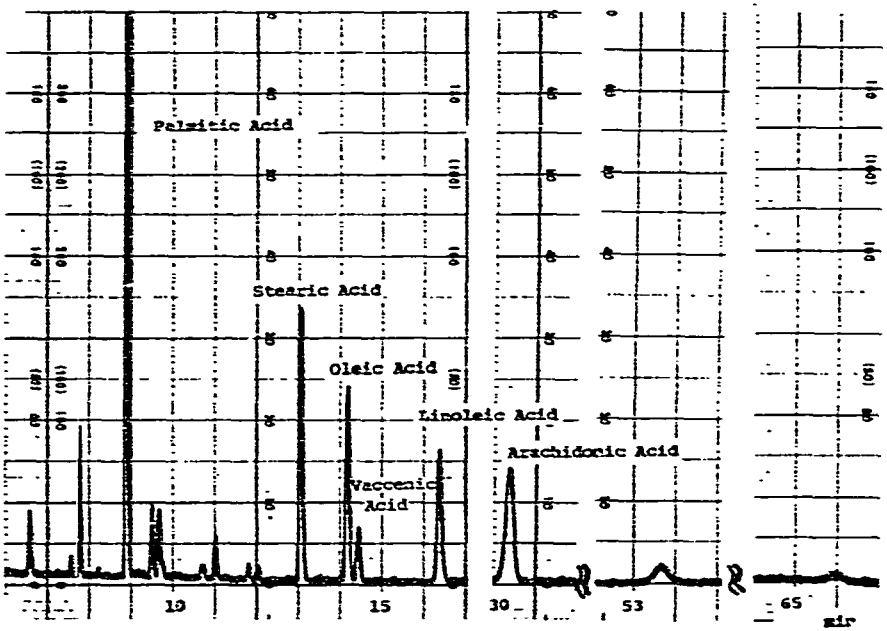

Fig. 5. Gas chromatogram of the methyl esters of the fatty acids associated with lung tissue phospholipids on a glass capillary column (40 m × 0.3 mm I.D.) coated with SS-4. The column was operated at 175°C with nitrogen as the carrier gas at a flow-rate of 1.4 ml/min. Injector and detector temperatures 250°C.

It can be concluded that a glass capillary column coated with the high polar stationary phase SS-4 is effective for the separation of geometric and positional isomers of unsaturated fatty acid methyl esters. Further investigations on the geometric and positional isomers of unsaturated fatty acid methyl esters are in progress.

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