Effect of Temperature on the Separation of Some Δ9-*cis-trans* Isomers of Methyl Esters of Fatty Acids by Open Tubular Gas Chromatography

J. HRIVŇÁK and L. SOJÁK, Comenius University, Institute of Chemistry, and
J. KRUPČÍK[1] and Y.P. DUCHESNE, Slovak Technical University, Chemical Faculty,
Bratislava, Czechoslovakia

ABSTRACT

The influence of temperature on the gas chromatographic separation of *cis-trans* isomers of the methyl esters of some monounsaturated fatty acids was studied on capillary columns coated with Apiezon L, BDS and DEGS. As far as methyl oleate and methyl elaidate are concerned, the separation is better at lower temperatures on Apiezon L (180-210 C) and at higher temperatures on polyester phases (BDS, DEGS; 150-180 C). The influence of temperature on the separation of *cis-trans* isomers on the three stationary phases under study is explained by the higher values of $\delta ECL/\delta t$ for *cis* isomers. The variation of the equivalent carbon chain length with temperature can be used for the identification of *cis-trans* isomers in natural mixtures.

Open-tubular gas liquid chromatography (GLC) of positional and geometric isomers of monounsaturated fatty acids is the most reliable method for characterizing every individual compound in natural mixtures. Its reliability is best on high efficiency capillary columns.

Separation of *cis-trans* isomers of methyl esters of monounsaturated fatty acids has been carried out on both nonpolar-coated (Apiezons) and polar-coated (polyesters, nitrile silicones) capillary columns (1-8). A good separation of *cis* and *trans* isomers is achieved on Apiezon L. On this phase methyl oleate (*cis* 18:1Δ9) elutes before methyl elaidate (*trans* 18:1Δ9), which is in agreement with their respective volatilities. However the analysis of biological material on Apiezon L does not give sufficient information about positional isomers of monounsaturated fatty acids (7,8). On polyester phases such problems do not appear, but the separation of *cis-trans* isomers is very poor; the interaction between the solute and the polyester phase causes reversal of the elution order, e.g., on the common types of polyesters (BDS, Reoplex 400, DEGS) as well as on nitrile silicone vaseline (XF-1150) methyl elaidate elutes a little before methyl oleate (1-8).

In our previous work we studied the effect of temperature on the separation of *cis-trans* isomers of C_6-C_{11} straight chain monounsaturated alkenes and determined the difference between the elution index temperature coefficients ($\delta I/\delta t$) of both *cis* and *trans* isomers of each of these alkenes upon separation on a high efficiency squalane-coated capillary column (12,14).

The purpose of this work is to investigate and interpret the influence of temperature on the separation of methyl esters of some *cis-trans* isomers of monounsaturated fatty acids using capillary columns coated with Apiezon L, BDS and DEGS; particular interest was given to the separation of methyl oleate and methyl elaidate.

EXPERIMENTAL PROCEDURES

Table I shows the instruments used and the operating conditions.

Materials

Oleic (*cis* 18:1Δ9) and elaidic (*trans* 18:1Δ9) acids were

[1]To whom all correspondence should be addressed.

TABLE I

Instruments Used and Operating Conditions

Conditions	Fractovap G 1		Perkin-Elmer 900
Column length, m	45	45	100
Column diameter, mm	0.25	0.25	0.25
Stationary phase	Apiezon L	DEGS	BDS
Temperature, C	180-210	150-180	160-180
N_2 pressure, kg/cm^2	1.0	1.0	2.0
Efficiency, theor. plates	60,000	80,000	100,000
Splitting ratio	1:200	1:200	1:250

TABLE II

Relative Retention Time of Methyl Oleate and Methyl Elaidate[a]

	Apiezon L		DEGS		BDS	
Temperature, C	r_1	$\delta r_1/10$ C	r_2	$\delta r_2/10$ C	r_2	$\delta r_2/10$ C
150			1.008	0.003		
160			1.011	0.004	1.012	
170			1.015	0.004		0.002
180	1.041	0.005	1.019		1.014	
190	1.036	0.005				
200	1.031	0.005				
210	1.026					

[a]Measured by open-tubular gas chromatography on Apiezon L, BDS and DEGS at different temperatures.

$$r_1 = \frac{t'_{R,trans\ 18:1\Delta 9}}{t'_{R,cis\ 18:1\Delta 9}}; \quad r_2 = \frac{t'_{R,cis\ 18:1\Delta 9}}{t'_{R,trans\ 18:1\Delta 9}}.$$

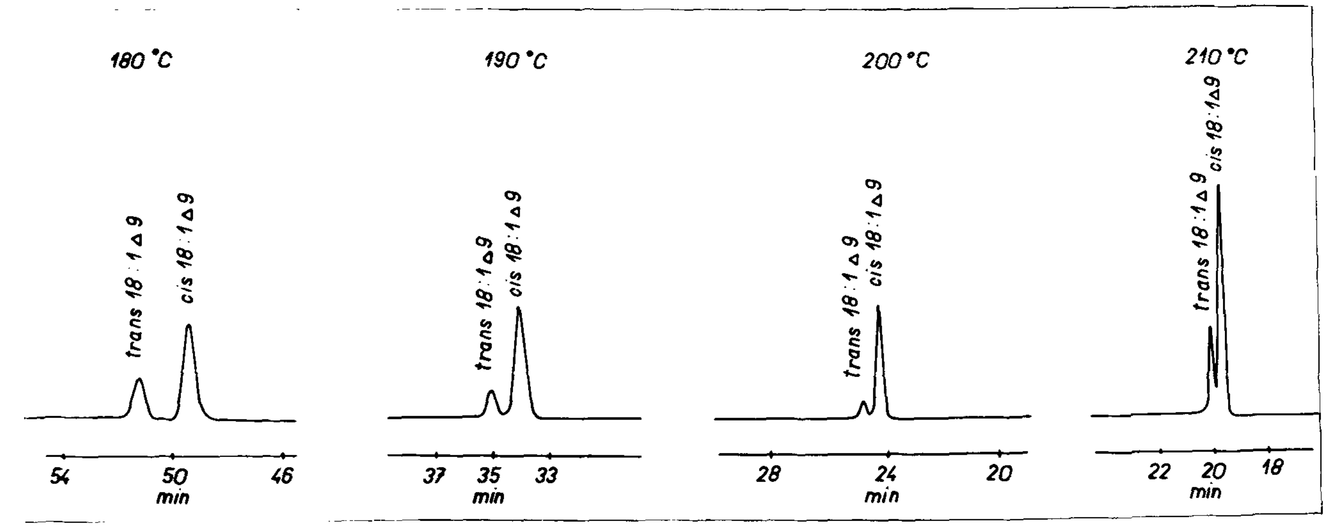

FIG. 1. Influence of temperature on the separation of methyl oleate (*cis* 18:1Δ9) and methyl elaidate (*trans* 18:1Δ9) by capillary gas chromatography on Apiezon L.

obtained from Lachema (Brno, Czechoslovakia); natural mixtures of fatty acids were isolated from the biochemical oxidation products of *n*-alkanes by the yeast microorganism *Candida lipolytica* (Slovnaft-Bratislava, Czechoslovakia). The lipids were extracted with methanol-chloroform 1:2. The acids were isolated by saponification of the lipid fraction followed by acidification with H_2SO_4 and extraction with diethyl ether (7). The methyl esters of these acids were prepared using diazomethane and then purified by thin layer chromatography (TLC) on silica gel. The monounsaturated esters were separated from the others by further TLC on $AgNO_3$-treated silica gel (10).

The isomeric identity of these unsaturated esters was established by TLC on $AgNO_3$-treated silica gel and separation of the constituents of each spot by open-tubular gas chromtography on Apiezon L and BDS.

Retention times are measured between the leading edge of the solvent peak and each peak maximum. All results are calculated from three measurements. Equivalent carbon chain length (ECL) values are calculated within a precision of 0.01 ECL unit.

RESULTS AND DISCUSSION

Figure 1 shows the influence of temperature on the separation of methyl oleate and methyl elaidate on Apiezon L.

It can be seen that the separation is better at low temperatures. The relative retention time $r_1 = \frac{t'_{R,\,trans\ 18:1\Delta 9}}{t'_{R,\,cis\ 18:1\Delta 9}}$

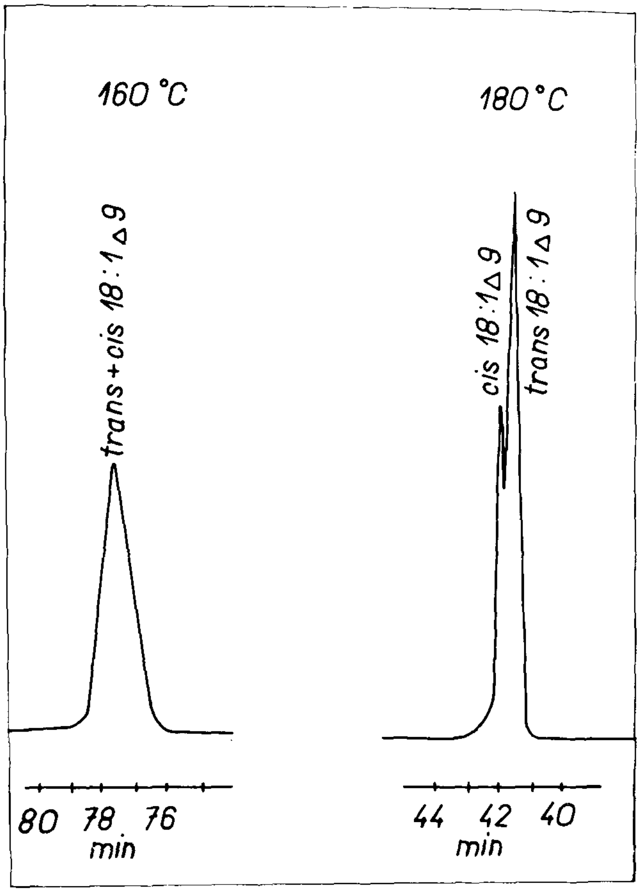

FIG. 2. Influence of temperature on the separation of methyl oleate (*cis* 18:1Δ9) and methyl elaidate (*trans* 18:1Δ9) by capillary gas chromatography on BDS.

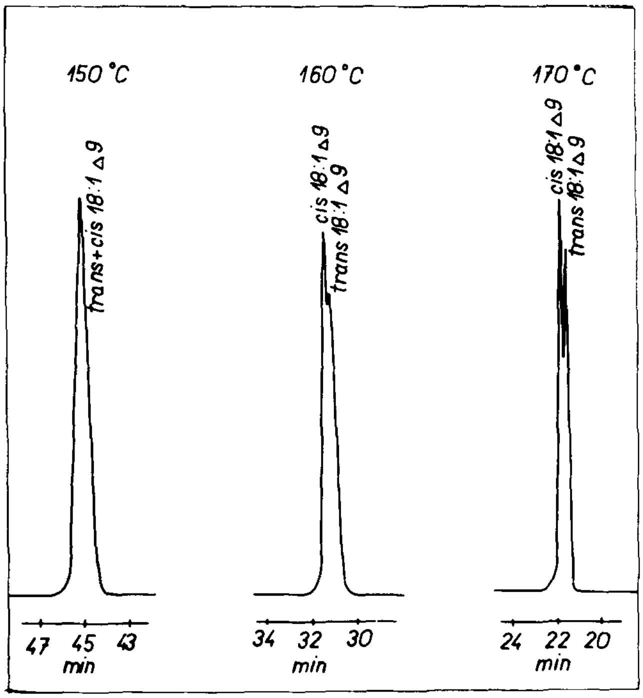

FIG. 3. Influence of temperature on the separation of methyl oleate (*cis* 18:1Δ9) and methyl elaidate (*trans* 18:1Δ9) by capillary gas chromatography on DEGS.

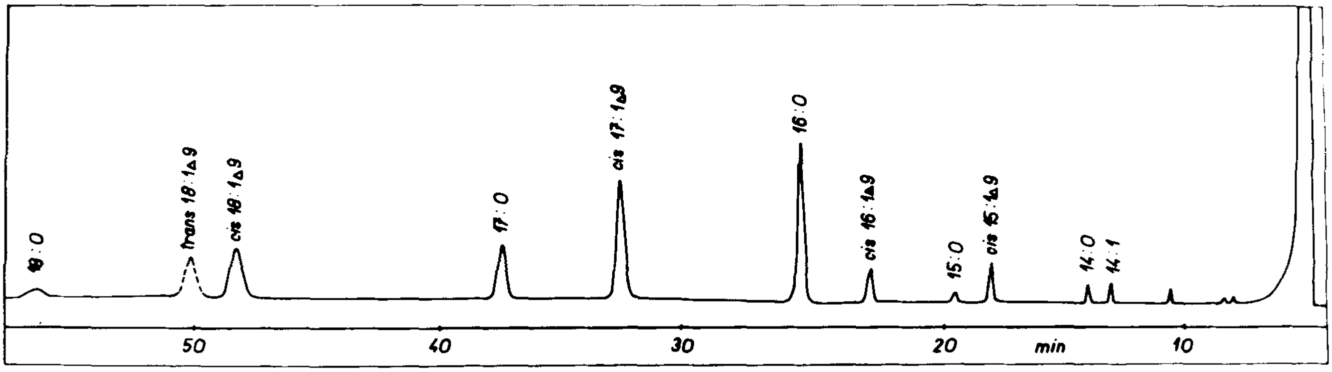

FIG. 4. Separation of the methyl esters of fatty acids isolated from biological material by capillary gas chromatography on Apiezon L at 180 C. Methyl elaidate (*trans* 18:1Δ9) (dotted peak) was added to the mixture.

decreases with increasing temperature (Table II), as the effect of temperature on the retention of the *cis* isomer is greater than on that of the *trans* isomer, as shown in Table III. A similar phenomenon has already been observed in the separation of *cis-trans* isomers of monounsaturated alkenes on squalane (12,14).

It is evident from Figure 1 and Table III that further increase of temperature will cause the *cis* and *trans* isomers to elute simultaneously or even in the inverse order.

Figure 2 shows the influence of temperature on the separation of methyl oleate and methyl elaidate on BDS. On this phase *cis* and *trans* isomers appear to elute in the inverse order, i.e., *trans* before *cis*, and their separation is better at high temperatures, as can be seen from the relative retention time $r_2 = \frac{t'_{R,\ cis\ 18:1\Delta 9}}{t'_{R\ trans\ 18:1\Delta 9}}$ depending on temperature (Table II).

Table III shows higher $\delta ECL/\delta t$ values for *cis* and *trans* isomers on BDS than on Apiezon L. The improvement of separation at higher temperatures is in agreement with the values of $\delta ECL/\delta t$ measured on BDS.

Figure 3 shows the influence of temperature on the separation of methyl oleate and methyl elaidate on DEGS. The elution order is the same as on BDS; the separation is better at higher temperatures, as can be seen from Table II.

Comparing the results for all three phases leads to the conclusion that the value of $\delta ECL/\delta t$ is influenced significantly by the temperature effect both on vapor pressure and on the interaction between the methyl esters and the stationary phase. Table III shows that $\delta ECL/\delta t$ increases with the polarity of the stationary phase for both *cis* and *trans* isomers, the difference $(\delta ECL/\delta t)_{cis}$ - $(\delta ECL/\delta t)_{trans}$ remaining roughly constant on polar phases (0.01-0.02).

Inversion of elution order, i.e., *cis* before *trans* is to be expected on BDS and DEGS at lower temperatures (see $\delta ECL/\delta t$ in Table III).

It can also be seen from Table III that on BDS and DEGS the increase of carbon chain length results in poorer separation of *cis* and *trans* isomers. A similar effect has already been observed on squalane for the separation of *cis-trans* C_6-C_{11} alkenes (12,14).

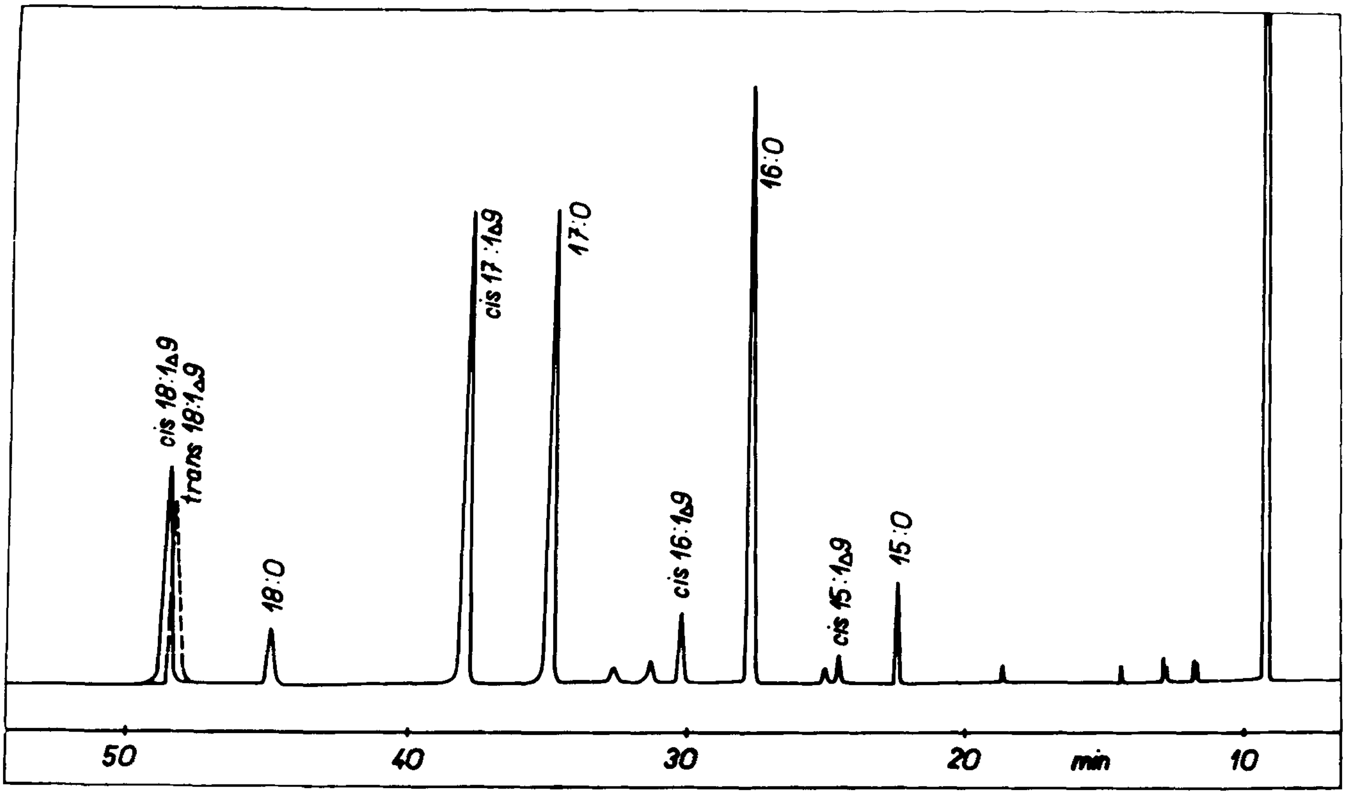

FIG. 5. Separation of the methyl esters of fatty acids isolated from biological material by capillary gas chromatography on BDS at 180 C. Methyl elaidate (*trans* 18:1Δ9) (dotted peak) was added to the mixture.

TABLE III

Values of ECL and δ ECL/δ *t* for Methyl Esters of Fatty Acids[a]

Methyl esters of	Apiezon L, 180 C		DEGS, 160 C		BDS, 160 C	
	ECL	δ ECL/20 C	ECL	δ ECL/20 C	ECL	δ ECL/20 C
15:0	15.00		15.00		15.00	
cis 15:1Δ9	14.67	0.02	15.45	0.08	15.25	0.05
16:0	16.00		16.00		16.00	
cis 16:1Δ9	15.71	0.03	16.49	0.08	16.35	0.04
trans 16:1Δ9			16.41	0.07	16.31	0.02
17:0	17.00		17.00		17.00	
cis 17:1Δ9	16.65	0.03	17.53	0.07	17.30	0.04
trans 17:1Δ9					17.26	0.03
18:0	18.00		18.00		18.00	
cis 18:1Δ9	17.61	0.03	18.35	0.09	18.27	0.05
trans 18:1Δ9	17.71	-0.01	18.33	0.07	18.24	0.03

[a]Measured by open-tubular gas chromatography on Apiezon L, BDS and DEGS.

The above results were used for the preliminary identification of monounsaturated fatty acids isolated from biological material. An example is shown on Figure 4 (Apiezon L; 180 C). Methyl elaidate (*trans* 18:1Δ9, dotted peak) was added to the mixture. The absence of *trans* isomers in the natural mixture was confirmed from two runs at different temperatures as well as by TLC on $AgNO_3$-impregnated silica gel (10).

The same mixture was analyzed on BDS (Fig. 5). The separation of *cis-trans* isomers is poorer here, but this phase makes it possible to separate positional isomers (7,9). Moreover, quantitative results can here be obtained with much less trouble than on Apiezon-L-coated capillary columns (Krupčik, unpublished results). The position of the double bond (Δ9) in all acids was established by two runs at different temperatures and confirmed by GLC analysis of the $KMnO_4$ oxidation products on BDS.

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