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GAS CHROMATOGRAPHIC RESOLUTION ON POLAR OPEN-TUBULAR COLUMNS OF SATURATED AND UNSATURATED WAX ESTER ISOMERS DIFFERING IN COMBINATIONS OF ACYL AND ALCOHOLIC GROUPS

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SUMMARY

The gas chromatographic resolution of saturated and unsaturated wax ester isomers having the same carbon number and the same degree of unsaturation, prepared from various fatty acids and fatty alcohols, was carried out on open-tubular glass capillary columns coated with a cyanosiloxane, SP-2340. Satisfactory resolution was achieved at lower column temperatures (up to 240°C) by introducing very small amounts of samples (several nanograms per component) into a short column (15 m), with hydrogen as the carrier gas. Equivalent chain lengths (ECLs) of wax ester isomers with 32–42 carbon atoms and with 1–6 double bonds are presented. The ECLs of polyunsaturated wax esters were significantly dependent on column temperature. The influence of column temperature on resolution is discussed.

INTRODUCTION

Wax esters occur widely in biological systems as complex mixtures of various combinations of fatty acids and fatty alcohols[1]. The gas chromatography (GC) of wax esters has usually been carried out on packed columns containing thermostable non-polar liquid stationary phases such as the carborane siloxane Dexil 300 or the methylsilicones SE-30 or OV-1, resulting in resolution based on their carbon numbers[2]. The recent development of highly polar cyanosiloxane stationary phases such as Silar 10C with moderate thermal stability has permitted the complete resolution of wax esters with 28–44 carbon atoms and 0–6 double bonds, based both on carbon number and degree of unsaturation, on packed columns[3]. The resolution of saturated and monoenoic wax esters with 32–36 carbon atoms based on carbon number and degree of unsaturation has also been observed in GC on non-polar open-tubular columns[4]. However, the satisfactory resolution of saturated and unsaturated wax ester isomers differing in combinations of acyl and alcoholic groups but having the same carbon number and same degree of unsaturation has not been achieved by GC on packed and open-tubular columns.

Polar open-tubular columns have been widely used for the GC analysis of fatty acid methyl esters, but their application to the GC analysis of higher molecular weight

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lipids is difficult because of their extremely short life at high temperatures. However, polar open-tubular columns with high efficiency and moderate thermal stability have become available recently. Myher and Kuksis[5] reported the GC resolution of diacylglycerols from natural glycerophospholipids with various combinations of acyl groups, which contain double bond positional isomers, on short open-tubular columns (10 m) coated with a cyanosiloxane, SP-2330. This result prompted us to study the GC resolution of wax ester isomers with various combinations of acyl and alcoholic groups on polar open-tubular columns.

A systematic investigation of the GC resolution of saturated and unsaturated wax ester isomers with 28–44 carbon atoms and 0–6 double bonds based on the differences in the ester group and double bond positions in the molecules was carried out on short open-tubular columns (15 m) coated with a cyanosiloxane, SP-2340, which is more polar than SP-2330. The GC behaviour of the wax esters on the columns is described in detail.

EXPERIMENTAL

*Wax esters**

The wax esters were prepared by transesterification of fatty acid methyl esters and fatty alcohols with sodium methoxide according to the method of Philips and Viswanathan[6], but the scale was reduced to about 1/20. Mixtures of fatty acid methyl esters (50 mg) and fatty alcohols (40 mg) with sodium methoxide (5 mg) were kept at 80°C in 0.5-ml glass vials with PTFE-linked screw-caps for 0.5–1 h with occasional stirring under nitrogen. The wax esters were separated from the reaction mixtures by thin-layer chromatography (TLC) on silica gel G (Merck, Darmstadt, F.R.G.) plates using *n*-hexane–benzene (1:1) as the developing solvent. The purity of the fatty acids and fatty alcohols used for wax ester synthesis was more than 95%. Saturated fatty acids with 8–22 carbon atoms were obtained from Tokyo Kasei (Tokyo, Japan); unsaturated fatty acids with 16–22 carbon atoms were prepared from plant and fish oils by argentation silicic acid column chromatography[7] and a conventional vacuum distillation method in our laboratory. Fatty acid mixtures from linseed and pollack liver oils were also used. Fatty alcohols were prepared from fatty acid methyl esters by reduction with lithium aluminium hydride. Wax ester standards, tetradecyl stearate (14:0–18:0), octadecyl palmitate (18:0–16:0), octadecyl stearate (18:0–18:0) and octadecyl oleate (18:0–18:1) were obtained from Applied Science Labs. (State College, PA, U.S.A.).

Gas chromatography

Open-tubular GC of the wax esters was carried out with a Shimadzu GC-7A gas chromatograph (Shimadzu Seisakusho, Kyoto, Japan) equipped with a dual hydrogen flame-ionization detector and a Quadrex glass capillary column (15 m × 0.25 mm I.D.) coated with SP-2340 (Supelco, Bellefonte, PA, U.S.A.). The column was

* Wax esters are designated as follows: alcoholic group–acyl group and/or total carbon number: degree of unsaturation. The double bond position of acyl or alcoholic groups is denoted in the form (*n*-*x*), where *n* is the total carbon number of the acyl or alcoholic groups and *x* the carbon number from the last double bond to the terminal methyl group.

connected to a capillary column holder (CLH-7, Shimadzu) with graphite ferrules without straightening either end. Hydrogen was used as the carrier gas at a flow-rate of 0.6 ml/min. All carrier gas pathways were made of glass tubes. A 1-μl volume of 0.01% (w/v) *n*-hexane solution per wax ester component was usually injected into the installation; the splitting ratio was 1:90. The attenuation was set at 1/2–1/8 × full sensitivity. The scavenger gas (H_2) and air flow-rates were 30 and 800 ml/min, respectively. To obtain maximum sensitivity, nitrogen in an amount approximately equal to that of the scavenger gas was passed to the flame-ionization detector. The detector and injector, in the same heater block, were maintained at 290°C. Isothermal analyses were carried out in the temperature range 180–240°C. Temperature-programmed analyses were usually carried out with heating from 200 to 240°C at 1°C/min. Measurements of peak area percentages, peak heights and retention times and various calculations from the retention data were carried out with a Chromatopac C-R2AX (Shimadzu).

RESULTS AND DISCUSSION

In our early work on the GC resolution of high-molecular-weight lipids on polar packed columns, we observed that their elution temperatures on Silar 10C were much lower than those on non-polar stationary phases[3,8–10]. This can be explained by the low affinity of non-polar lipids towards the polar stationary phase. In this study, short open-tubular columns (15 m) coated with SP-2340, which has a polarity similar to that of Silar 10C, were used.

Fig. 1 shows the resolution of fatty acid methyl esters prepared from pollack

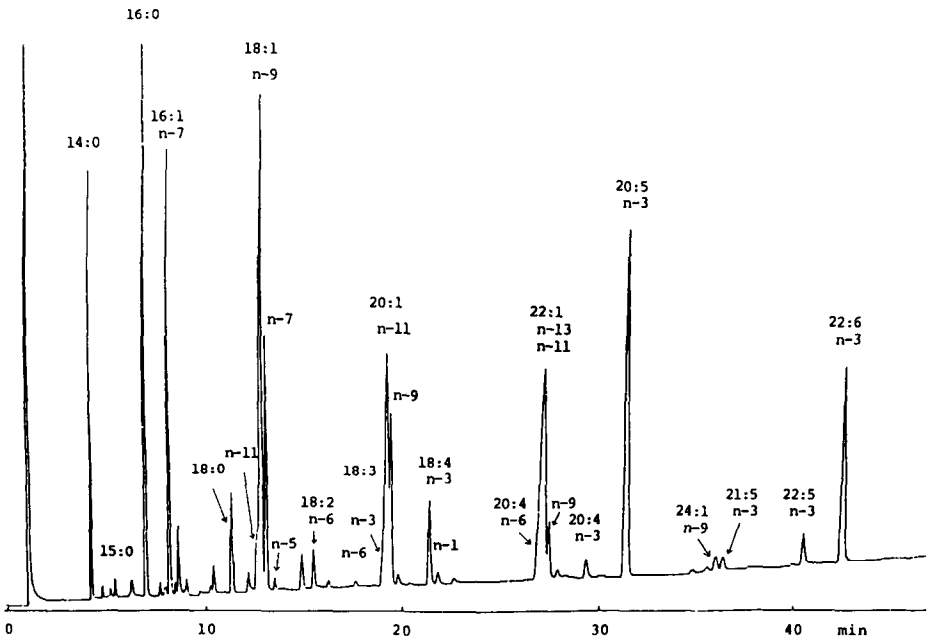

Fig. 1. GC resolution of fatty acid methyl esters from pollack liver oil on an open-tubular SP-2340 column. Temperature programmed from 140 to 190°C at 1°C/min.

liver oil on the open-tubular SP-2340 column employed for the wax ester resolution. In spite of the short column, most of the double bond positional isomers of monoenes and polyenes were resolved effectively, although the resolution between certain pairs of peaks was not complete. The acetates from fatty alcohols prepared by reduction of pollack liver oil fatty acid methyl esters also showed the same elution profile as the methyl esters. We obtained 54,000 theoretical plates for oleic acid (18:1 *n*-9) methyl ester at 160°C. To obtain complete resolution of the double bond positional isomers (*n*-11 and *n*-9) of 20:1 and 22:1, longer columns with more theoretical plates are required.

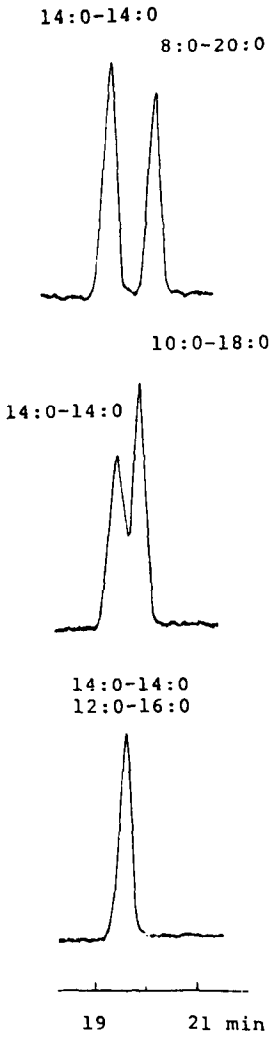

Fig. 2. Typical resolution of saturated wax ester isomers on an open-tubular SP-2340 column. Column temperature: 180°C.

Resolution of saturated wax ester isomers

Fig. 2 shows the typical resolution of saturated wax ester isomers differing in combinations of acyl and alcoholic groups but having the same carbon number. Resolution based on the difference in ester group positions was obtained at lower column temperatures such as 180°C by using the short open-tubular column coated with the highly polar stationary phase. Such resolution of saturated wax ester isomers by GC has not been reported. Although complete resolution was obtained for the pair of 14:0–14:0 and 8:0–20:0 isomers with ester groups in very different positions in the molecules, the resolution decreased considerably as the ester groups approached the centre of the molecule. Table I lists the peak separations[11], separation factors and differences in equivalent chain lengths (ECLs) for certain pairs of saturated wax ester isomers with 26–32 carbon atoms and differing in combinations of acyl and alcoholic groups. The best resolution of these isomer pairs on the column was obtained in the temperature range 180–190°C. At these temperatures, 30:0 and 32:0 isomers were eluted with leading peaks[11]. Sharp and symmetrical peaks of these isomers were obtained at higher temperatures, but the resolution decreased considerably, *e.g.*, the peak separation of the 16:0–16:0 and 10:0–22:0 isomers decreased to 65.9% at 200°C from 90.2% at 190°C; the peaks of the 12:0–18:0 and 16:0–14:0 isomers overlapped completely at 200°C. The retention times of wax ester isomers generally increased as the ester groups approached the end from the centre of the molecule. However, wax ester isomers differing by two carbon atoms at the ester group positions could not be resolved under the conditions used. In general, the resolution of wax ester isomers based on the difference in ester group positions is poor compared with that of the double bond positions. For example, the separation factor of 1.025 and difference in ECL of 0.08 of 10:0–18:0/14:0–14:0 at 180°C are

TABLE I

SEPARATION FACTORS (F), PEAK SEPARATIONS (δ) AND DIFFERENCES IN EQUIVALENT CHAIN LENGTHS (ΔECL) OF SATURATED WAX ESTER ISOMERS

Column temperature: 180°C for 26:0 and 28:0 and 190°C for 30:0 and 32:0.

Wax ester	*Isomer pair (alcoholic-acyl/alcoholic-acyl)*	*F*	*δ (%)*	*ΔECL**
26:0	16:0–10:0/12:0–14:0	1.021	17.5	0.05
	14:0–12:0/12:0–14:0	—**	—	—
28:0	12:0–16:0/14:0–14:0	—	—	—
	10:0–18:0/14:0–14:0	1.025	53.0	0.08
	8:0–20:0/14:0–14:0	1.042	100	0.15
30:0	14:0–16:0/16:0–14:0	—	—	—
	12:0–18:0/16:0–14:0	1.008	3.7	0.03
	10:0–20:0/16:0–14:0	1.030	82.9	0.11
	8:0–22:0/16:0–14:0	1.053	98.3	0.21
32:0	14:0–18:0/16:0–16:0	—	—	—
	12:0–20:0/16:0–16:0	1.016	19.7	0.06
	10:0–22:0/16:0–16:0	1.048	90.2	0.17

* ECL: 12:0 14:0, 26.00; 14:0 14:0, 28.00; 14:0–16:0, 30.00; 16:0–16:0, 32.00.

** Not resolved.

TABLE II

COMPARISON OF SEPARATION FACTORS OF WAX ESTERS AND FATTY ACID METHYL ESTERS

Wax esters [16:0–16:0, 16:0–18:0, 18:1 (*n*-9)–16:0, 18:2 (*n*-6)–16:0, 18:3 (*n*-3)–16:0] and fatty acids [16:0, 18:0, 18:1 (*n*-9), 18:2 (*n*-6), 18:3 (*n*-3)] were used to obtain these data.

Molecular species	*Wax ester*		*Fatty acid methyl ester*	
	200°C	*210°C*	*150°C*	*160°C*
Monoene/saturate*	1.103	1.102	1.171	1.160
Diene/monoene	1.169	1.161	1.285	1.264
Triene/diene	1.235	1.222	1.361	1.327
(CN+2)/CN**	1.611	1.567	1.711	1.597

* Separation factors of species having the same carbon number.
** Separation factors of saturated species. CN = carbon number.

approximately equal to those of 16:0–18:1 (*n*-9)/16:0–18:1 (*n*-7) at 200°C (see Table III).

Resolution of unsaturated wax ester isomers

Figs. 3 and 4 show the resolution of wax esters prepared from the fatty acid methyl esters of linseed and pollack liver oils, respectively, by transesterification with hexadecanol. The chromatograms are characterized by complete resolution based on the degree of unsaturation, partial resolution of monoenoic isomers differing in double bond position, elutions at remarkably low column temperatures and fairly stable baselines under high sensitivity with isothermal and temperature-programmed conditions. To obtain wax ester peaks of normal shape with satisfactory resolution, the introduction of very small amounts of samples into the column was necessary. In this study, several nanograms per component were usually introduced into the column through the split injection system. When larger amounts of samples were introduced, splitting and leading of peaks due to the overloading resulted, particularly with saturated and monoenoic wax esters. However, symmetrical peaks were observed when fatty acid methyl esters of 10-fold amounts of wax esters were introduced into the column (Fig. 1). The facile overloading of saturated components on open-tubular columns has also been observed in the GC of diacylglycerols on SP-2330[5]. The SP-2340 column used in this study had 11,000 theoretical plates for 18:0–18:0 at 220°C, which was about one-fifth of that for stearic acid (18:0) methyl ester at 160°C. It was also observed that the number of theoretical plates increased with increasing degree of unsaturation of wax esters having the same carbon numbers, *e.g.*, the plate numbers for 18:1 (*n*-9)–18:1 (*n*-9), 18:0–18:3 (*n*-3) and 18:1 (*n*-9)–18:3 (*n*-3) at 220°C were 15,000, 19,000 and 23,000, respectively. This explains the facile overloading of saturated and monoenoic wax esters observed on the column. The difference in the number of theoretical plates for fatty acid methyl esters and wax esters significantly affects the resolution of monoenoic isomers (see Figs. 3 and 4).

The elution pattern of wax esters prepared from pollack liver oil fatty acid

TABLE III

RELATIVE RETENTION DATA OF UNSATURATED WAX ESTER ISOMERS

The data for monoenoic wax esters were determined at 200 and 210°C; other data were at 220 and 230°C. RRTs of saturated wax esters used for ECL calculation at 200 and 210°C were as follows, respectively: 32:0 0.3552 0.3822; 34:0 0.6030 0.6239; 38:0 1.659 1.632. The values at 220 and 230°C are listed in Table IV.

Wax ester			*RRT**		*ECL***		*ΔECL****
	Alcoholic	*Acyl*	*220°C*	*230°C*	*220°C*	*230°C*	
32:1	14:0	18:1(*n*-9)	0.3967	0.4271	32.42	32.45	0.03
	16:0	16:1(*n*-7)	0.4090	0.4393	32.53	32.57	0.04
34:1	16:0	18:1(*n*-9)	0.6519	0.6788	34.31	34.36	0.05
	16:0	18:1(*n*-7)	0.6668	0.6941	34.40	34.45	0.05
	22:1(*n*-9)	12:0	0.6668	0.6941	34.40	34.45	0.05
36:1	16:0	20:1(*n*-11)	1.044	1.053	36.17	36.21	0.04
	16:0	20:1(*n*-9)	1.069	1.075	36.26	36.30	0.04
	18:1(*n*-9)	18:0	–	1.100	–	36.39	–
	22:1(*n*-9)	14:0	–	1.113	–	36.44	–
36:2	18:1(*n*-9)	18:1(*n*-9)	1.199	1.213	36.80	36.89	0.09
	18:1(*n*-9)	18:1(*n*-7)	1.220	1.236	36.87	36.98	0.11
	18:0	18:2(*n*-6)	1.257	1.264	37.00	37.09	0.09
38:2	18:1(*n*-9)	20:1(*n*-11)	1.808	1.791	38.57	38.68	0.11
	20:1(*n*-9)	18:1(*n*-9)	1.849	1.823	38.66	38.76	0.10
	20:0	18:2(*n*-6)	1.935	1.902	38.86	38.95	0.09
40:2	18:1(*n*-9)	22:1(*n*-11)	2.873	2.752	40.50	40.58	0.08
	22:1(*n*-9)	18:1(*n*-9)	2.962	2.831	40.62	40.71	0.09
	22:0	18:2(*n*-6)	3.105	2.975	40.82	40.92	0.10
36:3	18:1(*n*-9)	18:2(*n*-6)	1.403	1.409	37.48	37.59	0.11
	18:0	18:3(*n*-3)	1.499	1.501	37.77	37.88	0.11
40:3	22:1(*n*-9)	18:2(*n*-6)	3.340	3.210	41.12	41.25	0.13
	22:0	18:3(*n*-3)	3.583	3.413	41.40	41.51	0.11
36:4	18:0	18:4(*n*-3)	1.602	1.628	38.06	38.25	0.19
	18:1(*n*-9)	18:3(*n*-3)	1.668	1.689	38.23	38.41	0.19
38:4	18:0	20:4(*n*-6)	2.130	2.118	39.26	39.43	0.17
	20:0	18:4(*n*-3)	2.470	2.425	39.88	40.04	0.21
40:4	20:0	20:4(*n*-6)	3.295	3.182	41.06	41.21	0.15
	22:1(*n*-9)	18:3(*n*-3)	3.923	3.784	41.77	41.96	0.19
36:5	16:0	20:5(*n*-3)	1.669	1.700	38.24	38.44	0.20
	18:1(*n*-9)	18:4(*n*-3)	1.822	1.850	38.60	38.82	0.22
38:5	18:1(*n*-9)	20:4(*n*-6)	2.411	2.390	39.78	39.97	0.19
	18:0	20:5(*n*-3)	2.578	2.539	40.06	40.24	0.18
40:5	20:0	20:5(*n*-3)	3.900	3.762	41.75	41.94	0.19
	22:1(*n*-9)	18:4(*n*-3)	4.260	4.093	42.11	42.31	0.20
38:6	16:0	22:6(*n*-3)	2.772	2.734	40.35	40.55	0.20
	18:1(*n*-9)	20:5(*n*-3)	2.894	2.868	40.53	40.76	0.24
40:6	18:0	22:6(*n*-3)	4.089	3.981	41.94	42.18	0.24
	20:1(*n*-9)	20:5(*n*-3)	4.272	4.175	42.12	42.39	0.27
42:6	20:0	22:6(*n*-3)	6.328	6.003	43.78	43.99	0.21
	22:1(*n*-9)	20:5(*n*-3)	6.667	6.331	44.0	44.2	0.2

* RRT = retention time between the front of solvent deflection and peak maximum (18:0–18:0 = 1.000).

** ECL = equivalent chain length.

*** ΔECL = difference in ECLs at 220 and 230°C.

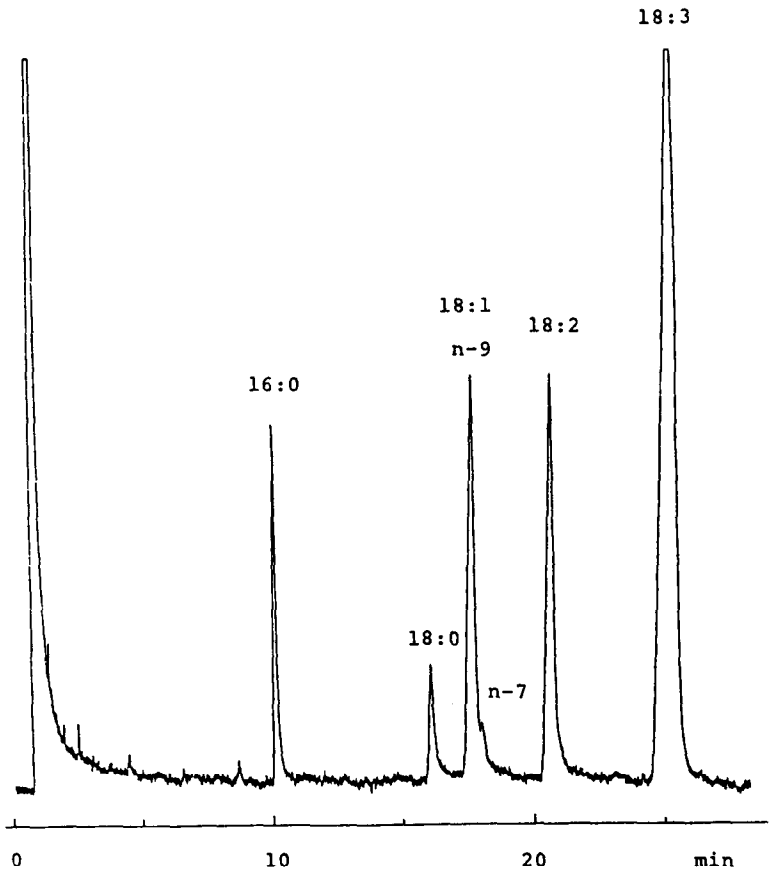

Fig. 3. GC resolution of wax esters prepared from linseed oil fatty acid methyl esters and hexadecanol on an open-tubular SP-2340 column. The acyl moieties are given above the peaks. Column temperature: 205°C.

methyl esters and hexadecanol differed slightly from that of the original methyl esters (Figs. 3 and 4). This is mainly due to the lower separation factors of wax ester homologues differing by two carbons and one double bond, in comparison with fatty acid methyl ester homologue (Table II). Such a difference in separation factors is caused by structural differences in the wax esters and fatty acid methyl esters and by the different column temperatures employed. The effect of column temperatures on the resolution on SP-2340 was remarkable, as described below.

Fig. 5 shows the typical resolution of various polyunsaturated wax ester isomers having different combinations of acyl and alcoholic groups but the same carbon number and the same degree of unsaturation. In spite of the high-molecular-weight components (40 carbons), complete resolution based on the difference in double bond positions in the molecules was achieved within 30 min for tri-, tetra-, penta- and hexaenoic isomers at 230°C. Dienoic isomers (40:2) were resolved completely at lower temperatures. Partial resolution of several pairs of such polyenoic wax ester isomers was observed in GC on polar packed columns[3]. The polar open-tubular columns,

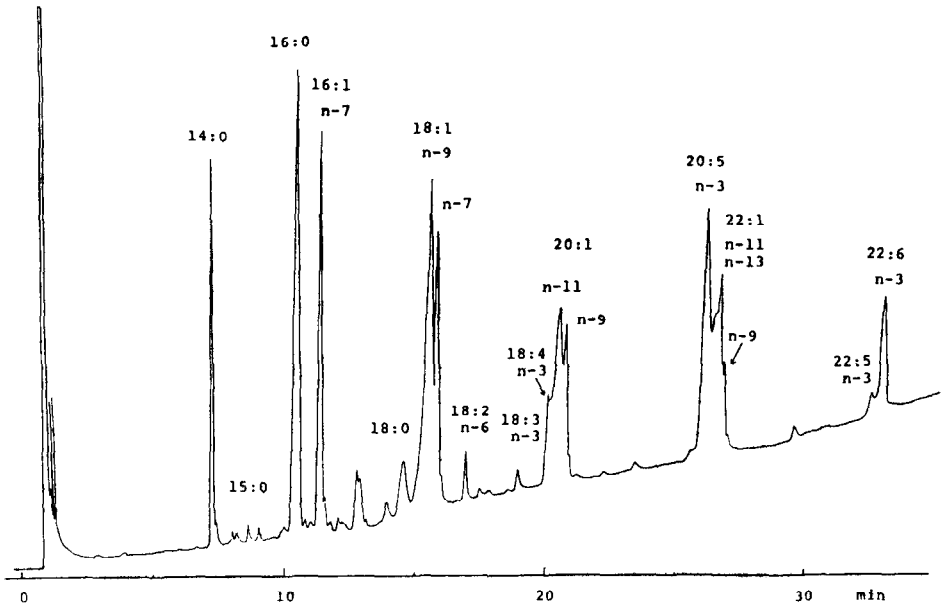

Fig. 4. GC resolution of wax esters prepared from pollack liver oil fatty acid methyl esters and hexadecanol on an open-tubular SP-2340 column. The acyl moieties are given above the peaks. Temperature programmed from 200 to 240°C at 1°C/min.

however, gave much better resolutions for all of the polyunsaturated wax ester isomers prepared in this study with shorter retention times (see Table III).

Table III lists the relative retention data for all the unsaturated wax ester isomers. The ECL widely used for fatty acid methyl esters is based on the linear relationship between the logarithm of the retention time of normal saturated methyl esters and their carbon number on packed columns[12]. However, a detailed investigation using open-tubular columns 40–50 m long indicated that the plots of the adjusted retention times against carbon number of fatty acid methyl esters was not linear, but could be best approximated by second-order equations[13,14]. In this study, using short open-tubular columns, the plot of the retention time against carbon number of saturated wax esters with 22–44 carbon atoms showed a slightly positive deviation from a straight line. Table IV compares the carbon numbers calculated on the basis of a straight line and a parabola for saturated wax esters with 24–44 carbon atoms. The data show that the deviation approximates a parabola rather than a straight line, although the quadratic relationship is not apparent. Thus, the ECLs of unsaturated wax ester isomers shown in Table III were calculated from the logarithm of the relative retention times of the neighbouring saturated homologous pairs with even carbon numbers. Such non-linearity phenomena have also been observed in the packed column GC of mono-, di- and triacylglycerols on highly polar Silar 10C[8,10] and of triacylglycerols on non-polar JXR[15].

The data in Table III can be used to determine the relationship for the elution order of unsaturated wax ester isomers on open-tubular SP-2340 columns such that the retention times of the isomers increased with the approach of double bonds to

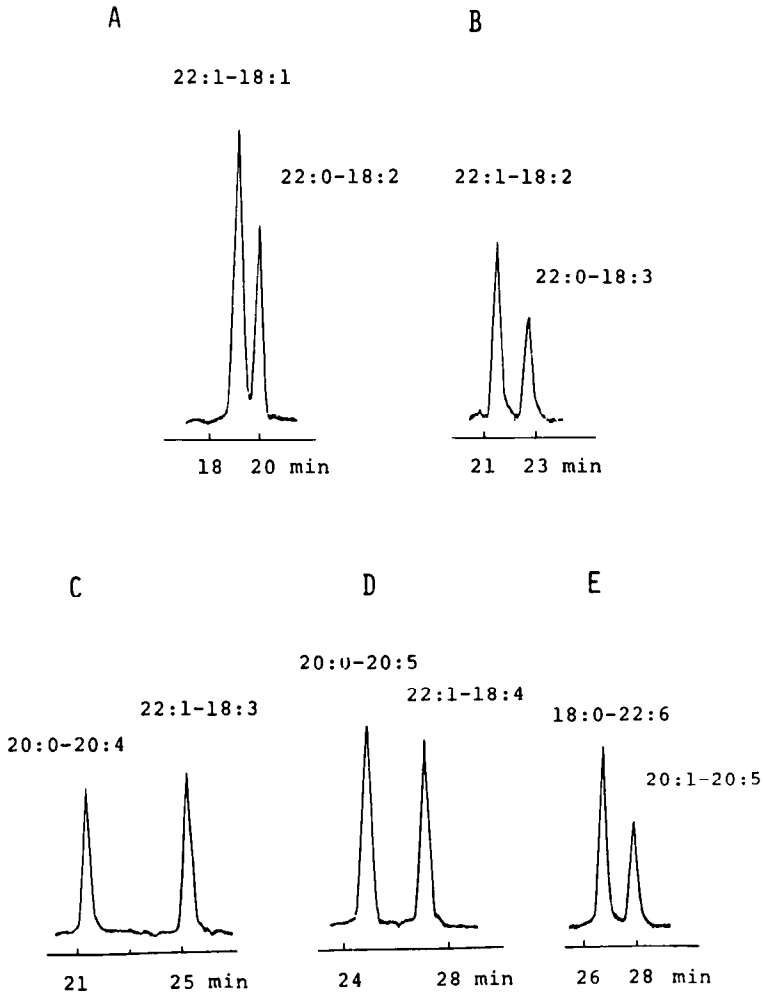

Fig. 5. Typical resolution of polyunsaturated wax ester isomers on an open-tubular SP-2340 column. A, 40:2; B, 40:3; C, 40:4; D, 40:5; E, 40:6. Column temperature: 230°C.

the end of the molecule. The shifts in retention times, depending on the difference in the ester group positions in the molecule, were not observed for the wax ester isomers listed in Table III under the conditions used.

Effects of column temperature on resolution

Wax esters whose ECL difference is more than 0.16 were resolved almost completely on open-tubular SP-2340 columns (Table III). However, when the difference was less than 0.06 hardly any resolution was possible at the temperatures in Table III. However, some critical pairs with different degrees of unsaturation could be resolved easily by an appropriate selection of column temperature. Fig. 6 shows the typical resolution of overlapping peak components of different degrees of unsaturation by such a selection of column temperature. The saturate–tetraene pair, 18:0–

TABLE IV

COMPARISON OF CARBON NUMBERS (CN) FOR SATURATED WAX ESTERS CALCULATED BY LINEAR AND QUADRATIC METHODS

The RRTs of 30:0 and 38:0 were used as reference points in the linear method; the RRTs of 30:0, 34:0 and 38:0 were used as reference points in the quadratic method.

Wax ester			*Column temperature*					
	Alcoholic	*Acyl*	*220°C*			*230°C*		
			*RRT**	*Linear***	*Quadratic****	*RRT**	*Linear§*	*Quadratic§§*
24:0	12:0	12:0	0.076181	24.40	24.29	0.090244	24.47	24.38
26:0	14:0	12:0	0.11447	26.22	26.16	0.13163	26.27	26.14
28:0	14:0	14:0	0.17214	28.06	28.03	0.19279	28.09	28.03
30:0	14:0	16:0	0.26547	30.00	30.00	0.28785	30.00	30.00
32:0	16:0	16:0	0.40497	31.90	31.91	0.42845	31.90	31.93
34:0	16:0	18:0	0.64394	33.98	34.00	0.65930	33.95	34.00
36:0	18:0	18:0	1.0000	35.95	35.97	1.0000	35.94	35.98
38:0	18:0	20:0	1.5777	38.00	38.00	1.5395	38.00	38.00
40:0	20:0	20:0	2.5419	40.14	40.11	2.4052	40.13	40.07
42:0	22:0	20:0	4.1479	42.34	42.27	3.8178	42.33	42.19
44:0	22:0	22:0	6.6597	44.46	44.34	6.0121	44.50	44.24

* RRT = retention time between the front of solvent deflection and the peak maximum (36:0 = 1.0000).

** $\text{Log(RRT)} = 0.096751(\text{CN}) - 3.4785$.

*** $\text{Log(RRT)} = 0.00013591(\text{CN})^2 + 0.087510(\text{CN}) - 3.3236$.

§ $\text{Log(RRT)} = 0.091027(\text{CN}) - 3.2716$.

§§ $\text{Log(RRT)} = 0.00026188(\text{CN})^2 + 0.073219(\text{CN}) - 2.9731$.

20:0 and 18:0–18:4 (*n*-3), overlapping at 220°C, was resolved dramatically by changing the column temperature slightly. The tetraene eluted faster than the saturate at lower temperatures, whereas the reverse occurred at higher temperatures. The ECL of the 18:0–18:4 (*n*-3) isomer increased considerably, from 37.77 at 210°C to 38.39 at 240°C. Such a dramatic resolution by the changing column temperature was noted for saturate–polyene pairs. The slow elution of the tetraene at higher temperatures suggests an increment in the polarity of the cyanosiloxane SP-2340 stationary phase. This can be attributed to conformational changes in the polymers, having bulky polar side-chains, with increasing thermal energy, as described for GC on the cyanosiloxane XE-60[16]. However, wax ester pairs with nearly the same degree of unsaturation, such as 18:1 (*n*-9)–18:3 (*n*-3) and 16:0–20:5 (*n*-3), were not resolved as effectively as saturate–polyene pairs by changing the column temperature. This is due to the different temperature effects for saturated and polyunsaturated wax esters on SP-2340. Thus, wax esters with the same degree of unsaturation have a similar temperature effect on SP-2340. The difference in ECLs at 220 and 230°C shown in the last column in Table III indicates that the temperature effect generally increases with increasing degree of unsaturation of the wax esters. This was also observed in the packed column GC of wax esters on Silar 10C[3]. In general, the temperature effect is remarkable in the GC of wax and steryl esters of lesser polarity than fatty acid methyl esters, tri-

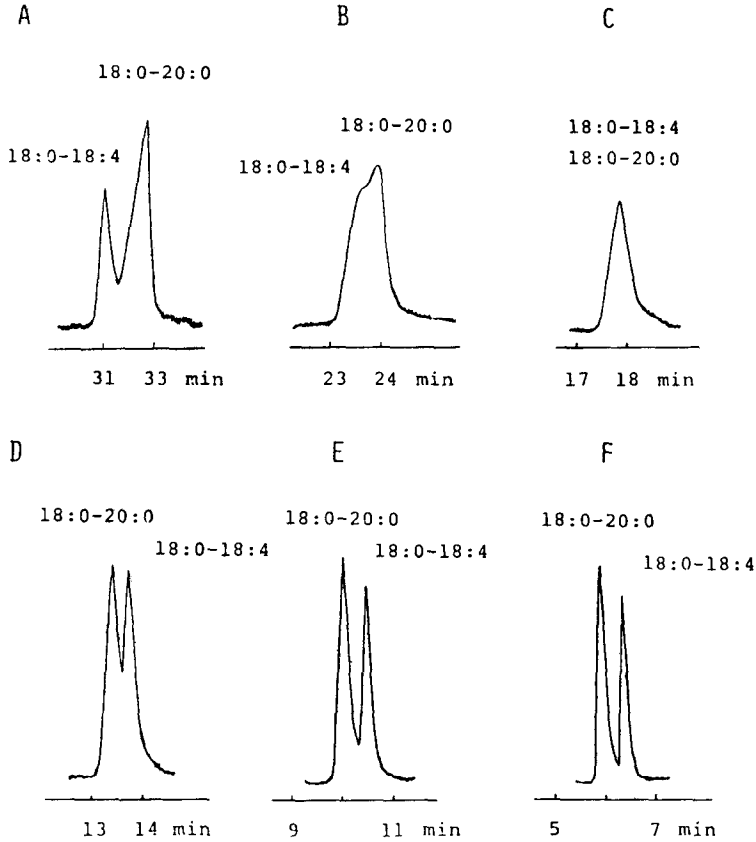

Fig. 6. Typical resolution of overlapping peak components by changing the column temperature on an open-tubular SP-2340 column. A, 210°C; B, 215°C; C, 220°C; D, 225°C; E, 230°C; F, 240°C.

TABLE V

COMPARISON OF WAX ESTER COMPOSITIONS OBTAINED WITH POLAR OPEN-TUBULAR AND PACKED COLUMNS

Mean peak area (%) ± S.D. of five analyses.

*Wax ester**		*Column*	
Alcoholic	*Acyl*	*Open-tubular***	*Packed****
16:0	16:0	6.33 ± 0.33	6.25 ± 0.20
16:0	18:0	2.97 ± 0.22	3.13 ± 0.18
16:0	18:1(*n*-9)	14.54 ± 0.33	15.44 ± 0.07
16:0	18:1(*n*-7)	0.97 ± 0.12	
16:0	18:2(*n*-6)	16.02 ± 0.60	16.01 ± 0.06
16:0	18:3(*n*-3)	59.17 ± 0.52	59.17 ± 0.35

* Hexadecyl esters of linseed oil fatty acids (see Fig. 3).

** Column temperature: 205°C.

*** 5% Silar 10C (1.5 m long) on 100–120-mesh Gas-Chrom Q. Column temperature: 240°C. Other GC conditions as given in a previous paper[3].

acylglycerols and mono- and diacylglycerol derivatives, and increases with increasing polarity of cyanosiloxane stationary phases[10].

Quantitative analysis

Table V compares the compositions of wax esters prepared from linseed oil fatty acid methyl esters and hexadecanol obtained by GC on polar open-tubular and packed columns. The polar packed column gave five peaks according to the degree of unsaturation and carbon number, as reported in a previous paper[3]. The good agreement between the peak area percentages obtained with both polar open-tubular and packed columns indicates the reliability of GC on open-tubular SP-2340 columns for the quantitative analysis of wax esters.

The open-tubular columns employed in this study could be used for approximately 500 h at temperatures below 240°C, the maximum temperature recommended, although the column efficiency decreased gradually. A small amount of the 16:0–18:1 (*n*-7) isomer was resolved from the 16:0–18:1 (*n*-9) isomer on the newer columns, but was not resolved on the older columns.

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