Separation of mono- and diglycerides by gas–liquid chromatography

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ABSTRACT The parameters affecting the separation and quantification of trimethylsilyl ethers of mono- and diglycerides have been investigated by gas–liquid chromatography with QF-1 and SE-30 as stationary phases and a flame ionization detector. Results have been compared with those obtained earlier for triglycerides.

The isothermal characteristics of a range of trimethylsilyl ethers of mono- and diglycerides on both stationary phases showed that log retention volume was directly proportional to carbon number and inversely proportional to absolute temperature. However, glyceride derivatives with lower carbon numbers deviated from these relationships.

By using various rates of programmed temperature rise, we have determined the elution temperatures (Kelvin scale) of the mono- and diglyceride trimethylsilyl ethers relative to that of glycerol trilaurate. The "carbon equivalent of a trimethylsilyl group" is defined and shown to be useful in comparing the chromatographic properties of different glyceride classes.

Weight and molar correction factors have been obtained and used to analyze diglycerides derived from egg and bovine brain lecithins.

SUPPLEMENTARY KEY WORDS trimethylsilylation · isothermal · temperature programming · elution temperatures · correction factors

EARLY WORKERS employed acetate derivatives for the GLC of mono- and diglycerides (1), and McInnes, Tattrie, and Kates (2) used allyl esters or isopropylidene derivatives to separate monoglycerides. More recently, Kuksis and Breckenridge (3) have resolved dibutyrate and benzylidene derivatives of isomeric glycerol 1- and 2-monopalmitates.

The first separation and quantification of TMS derivatives of long-chain ($>C_{10}$) monoglycerides by isothermal GLC was reported by Wood, Raju, and Reiser (4). Temperature-programmed GLC was used (5) to separate TMS derivatives of mono- (C_{12}–C_{18}) and diglycerides (C_{12}–C_{24}), though the technique was not claimed to be quantitative. In all cases in which positional isomers of TMS ethers of mono- and diglycerides have been separated (4–6), the 2-monoglycerides and 1,2-diglycerides were eluted before the corresponding isomers.

Recently, Tallent, Kleiman, and Cope (7) and Tallent and Kleiman (8) have made quantitative GLC studies of the TMS ethers of products of partial lipolysis of triglycerides. The only attempt to use correction factors (f_w) to quantify GLC analysis of TMS ethers of mono- and diglycerides was that of Sahasrabudhe and Legari (9).

Except for the fact that an almost linear relationship was found between retention temperature and carbon number (5), few qualitative parameters defining the behavior on GLC of TMS ethers of mono- and diglycerides have been reported. We have therefore extended our studies on the parameters affecting the separation of triglycerides by GLC on SE-30 (methylpolysiloxy gum) and QF-1 (methyl fluoroalkyl silicone) (10) to the TMS ethers of a wide range of mono- (C_2–C_{18}) and diglycerides (C_4–C_{36}). Using glycerol tricaprylate as a reference compound, we have correlated the behavior of these TMS ethers on GLC with that of triglycerides. For comparison, we have also investigated quantification by means of the SE-30 column, which gave excellent separation of triglycerides.

A preliminary report of some of these data was presented at a symposium organized by the Gas Chromatography Discussion Groups of the Institute of Petroleum, 22 April 1966.

Abbreviations: GLC, gas–liquid chromatography; TMS, trimethylsilyl; V_r, retention volume; C, number of carbon atoms in a fatty acid or total number of *acyl* carbon atoms in a mono-, di-, or triglyceride; C_2–C_{36}, glycerides with a total number of *acyl* carbons of 2–36.

MATERIALS

GLC column packings and gases used have been previously described (10).

Glycerol 1-monopalmitate, glycerol 1-monooleate, glycerol dioleate, and glycerol distearate (all 99% pure) were purchased from Sigma Chemical Co., London, England. A sample of glycerol 1-monostearate was kindly supplied by Dr. G. Hübscher of this Department. Commercial samples of glycerol laurates and glycerol myristates were found to contain mono-, di-, and triglycerides. Mono- and diglycerides were obtained from these commercial samples by thick-layer chromatography as follows. 20 × 20 cm chromatoplates were coated with a 1 mm layer of Silica Gel G (Mackerey, Nagel & Co., Düren, Germany) containing boric acid (11), and developed in either diethyl ether–*n*-hexane 40:60 or chloroform–acetone (various compositions from 90:10 to 75:25). Samples of glycerol acetates (Hopkin and Williams Ltd., Chadwell Heath, Essex), glycerol caprylates (Sir Thomas and Arthur Wardle & Co., Leek, Staffordshire), and glycerol palmitates (Sigma Chemical Co.) were also separated by thick-layer chromatography to give mono- and diglycerides. Dr. C. Barrett, Unilever Research Laboratory, The Frythe, Welwyn, Herts., generously provided samples of glycerol 1,3-dilaurate, glycerol 1,2- and 1,3-dipalmitates, and glycerol 1-palmitate 3-stearate. We synthesized glycerol mono- and di- butyrate, caproate, and caprate from glycerol plus 99% pure fatty acid (Sigma Chemical Co.) using trifluoroacetic anhydride (12). Thick-layer chromatography was used for separation and purification. The methods used to determine the purity of mono- and diglycerides were those we had employed for triglycerides (10). Purity of the products was in the range 95–99% except for glycerol dimyristates (about 90%) where impurities were glycerol laurate myristate (7.5%) and glycerol myristate palmitate (2.5%). Diglycerides obtained from egg and bovine brain lecithins were the gifts of G. White and Dr. D. White of this Department.

METHODS

GLC methods and conditions were the same as those used in studies on triglycerides (10), except that the temperature range was 120–325°C programmed at rates of actual temperature rise of 1.85, 3.71, and 5.56°C/min.

To prepare TMS ethers, we dissolved mono- and diglycerides in chloroform (20 mg/ml) and allowed 20 μl portions to react with 5 μl of bis(trimethylsilyl)acetamide for 5 min at room temperature. 2.5 μl of the resultant solution was then injected onto the GLC columns.

RESULTS

Silylation

To judge by both GLC and thin-layer chromatography, monoglyceride silylation by bis(trimethylsilyl)acetamide was complete in 1 min, but up to 5 min was required for diglyceride silylation. When hexamethyldisilazane plus trimethylchlorosilane were used, silylation of both mono- and diglycerides was complete within 5 min.

Both free and partially silylated saturated diglyceride mixtures (C_4–C_{36}) were analyzed by GLC on SE-30. Extraneous peaks were always observed in the triglyceride region even though the loadings used were not large (50 μg of the mixture). Similar peaks were observed when free monoglycerides were applied, though only at high loads (220 μg of a mixture of C_2–C_{18}).

Qualitative GLC Characteristics

Isothermal Analysis. As with triglycerides (10), mono- and diglyceride TMS ethers were analyzed at several temperatures. Log V_r was directly proportional to C at each temperature used on both SE-30 or QF-1 (Fig. 1). However, it was necessary to apply two or three samples of the monoglyceride TMS ethers to either column at each temperature used between 130 and 180°C before consistent results were obtained. Fig. 1a and c also show a partial separation of some 1- and 2-monoglyceride TMS ethers. Fig. 2 shows that the relationship $\log V_r = a + b/T$ (where a and b are constants and T is in °K) holds for both classes of glyceride TMS ethers.

Column efficiencies in terms of ΔC (the minimum carbon number difference between two mono- or diglyceride TMS ethers that can be completely resolved) and theoretical plate numbers for both mono- and diglyceride TMS ethers were determined. Under conditions similar to those used previously for triglycerides, similar values were obtained.

Temperature-Programmed Analysis. Relative elution temperatures (T_{RE}) for mono- and diglyceride TMS ethers were determined relative to glycerol trilaurate (C_{36}) so that a comparison with values determined for triglycerides (10) might be readily made. The results are shown in Table 1 (monoglyceride TMS ethers) and Table 2 (diglyceride TMS ethers).

It was of interest to see if the TMS group conferred the same chromatographic properties irrespective of the type of glyceride or molecular weight. A simple way to determine this effect is to define "the carbon equivalent of a TMS group" (ΔC_{TMS}) as the difference, per TMS group, in carbon number between the mono- or diglyceride TMS ether and the triglyceride with the same T_{RE} under the same conditions of temperature programming. The values obtained for the range of mono- and diglycerides used in this study on SE-30 at 3.71°C/min and on QF-1

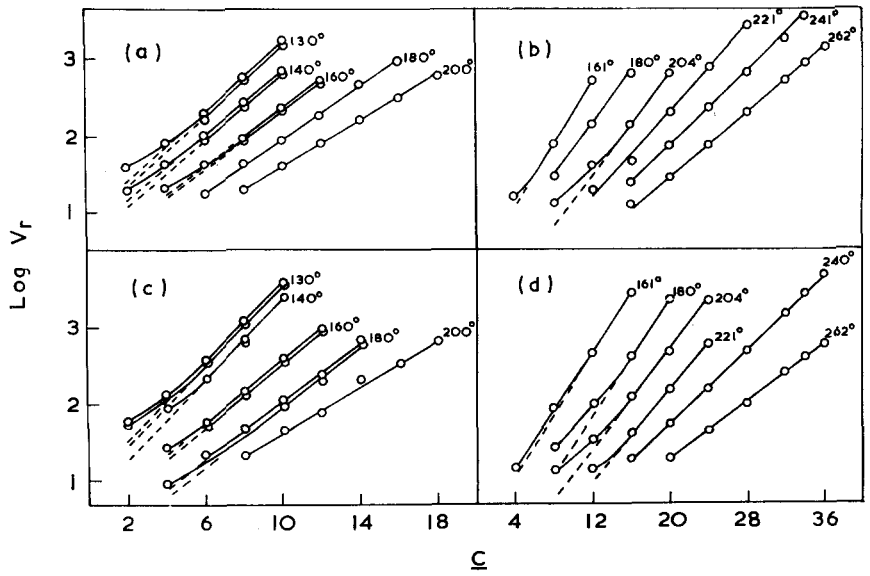

FIG. 1. Relationship of log V_r and C at various temperatures (in °C) for saturated mono- and diglyceride TMS ethers: (a) monoglyceride TMS ethers on SE-30, (b) diglyceride TMS ethers on SE-30, (c) monoglyceride TMS ethers on QF-1, and (d) diglyceride TMS ethers on QF-1. Duplicate samples were chromatographed at each temperature, though two or three samples of monoglyceride TMS ethers had to be applied initially to obtain consistent results. Monoglyceride mixture composition (weight %): C_2 9.0, C_4 4.7, C_6 18.9, C_8 10.9, C_{10} 9.4, C_{12} 13.7, C_{14} 4.6, C_{16} 9.9, and C_{18} 18.9. Diglyceride sample composition as in Table 2. Reproducibility was ±2–3% for monoglyceride TMS ethers and ±1% for diglyceride TMS ethers. Where monoglyceride TMS ethers were partially separated as shown by parallel plots in (a) and (c), the 1-isomer was eluted first.

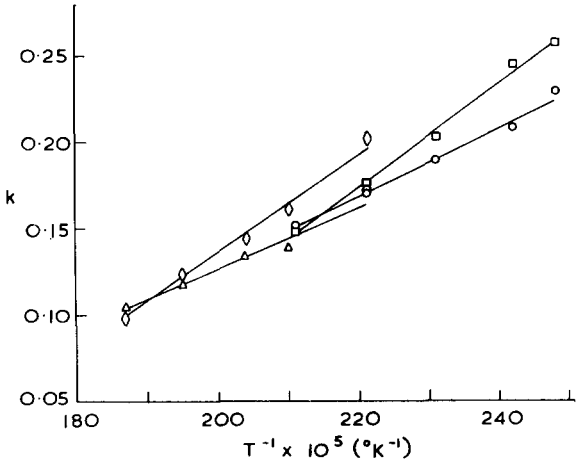

FIG. 2. Relationship between k and T^{-1} for saturated mono- and diglyceride TMS ethers. ○, monoglyceride TMS ethers on SE-30; □, monoglyceride TMS ethers on QF-1; △, diglyceride TMS ethers on SE-30; ◇, diglyceride TMS ethers on QF-1. k is the constant of the relationship log $V_r = kC$, obtained from Fig. 1:

at 5.56°C/min are shown in Table 3. Fig. 3 illustrates how these values were obtained.

Table 4 gives values for T_{RE} and ΔC_{OH} for some free diglycerides on SE-30 with temperature programming of 5.56°C/min. ΔC_{OH} is determined in a manner analogous to that for ΔC_{TMS} and is defined as "the difference in carbon number between the free diglyceride and the triglyceride with the same T_{RE} under the same conditions of temperature programming."

Quantitative Characteristics

The SE-30 column used to determine the quantitative characteristics of monoglyceride TMS ethers was that employed in our triglyceride studies. For quantitative characterization of diglyceride TMS ethers, a newly prepared column of SE-30 was used, except where otherwise stated.

Detector response to column loading was linear with loads up to 56 μg of monoglyceride TMS ether and to 54 μg of diglyceride TMS ether.

Values of f_w (a weight correction factor to correct values to correspond to the free glyceride) have been determined by analyzing the TMS ethers of saturated monoglycerides (Table 5) and diglycerides (Table 6). In neither case could a smooth curve of f_w versus C be fitted to the plots. The values of f_w for glycerol mono- and dioleates were slightly lower than those for glycerol mono- and distearates, respectively.

Application to Natural Samples

Table 7 gives the composition of the diglyceride TMS ethers derived from egg and bovine brain lecithin as determined by GLC on SE-30.

TABLE 1 RELATIVE ELUTION TEMPERATURES (T_{RE})* OF TMS ETHERS OF SATURATED MONOGLYCERIDES C_2–C_{18} ON QF-1 AND SE-30

Monoglyceride (Carbon Number)	Corrected Program Rate (°C/min)					
	1.85		3.71		5.56	
	QF-1	SE-30	QF-1	SE-30	QF-1	SE-30
2	0.448 ± 0.002	0.428 ± 0.002	0.464 ± 0.003	0.419 ± 0.000 0.427 ± 0.008	0.445 ± 0.009 0.450 ± 0.012	0.420 ± 0.001
4	0.481 ± 0.002	0.449 ± 0.002	0.505 ± 0.003	0.455 ± 0.016	0.480 ± 0.009 0.494 ± 0.018	0.448 ± 0.000
6	0.512† 0.515 ± 0.000	0.474 ± 0.000 0.480 ± 0.002	0.536 ± 0.002 0.543 ± 0.002	0.476 ± 0.000 0.495 ± 0.020	0.529 ± 0.005 0.544 ± 0.006	0.504 ± 0.003 0.507 ± 0.003
8	0.566† 0.576 ± 0.002	0.543 ± 0.005 0.549 ± 0.005	0.594 ± 0.001 0.598 ± 0.001	0.531 ± 0.002 0.550 ± 0.020	0.598† 0.605 ± 0.004	0.534 ± 0.003 0.540 ± 0.003
10	0.624† 0.632 ± 0.004	0.607 ± 0.000 0.614 ± 0.000	0.647 ± 0.002 0.650 ± 0.001	0.583† 0.604 ± 0.018	0.657 ± 0.002	0.590 ± 0.001 0.597 ± 0.001
12	0.672 ± 0.004	0.636 ± 0.004	0.692 ± 0.001	0.661 ± 0.004	0.693 ± 0.004	0.651 ± 0.008
14	0.714 ± 0.006	0.695 ± 0.000	0.731 ± 0.002	0.699 ± 0.004	0.735 ± 0.000	0.704 ± 0.001
16	0.771 ± 0.009	0.730 ± 0.004	0.779 ± 0.001	0.741 ± 0.006	0.778 ± 0.000	0.742 ± 0.001
18	0.787 ± 0.002	0.763 ± 0.005	0.810 ± 0.001	0.778 ± 0.001	0.808 ± 0.000	0.780 ± 0.001

Results were in duplicate, with absolute deviations given, except for SE-30 at 3.71°C/min and QF-1 at 5.56°C/min, where results were triplicate with standard deviations given. Sample composition as given in Fig. 1 with 2.5–12.5 μl of solution injected. Where two values of T_{RE} are given, the lesser value refers to the 1-isomer.

* Glycerol trilaurate has been assigned a T_{RE} of 1.00 for direct comparison of results with those obtained with diglyceride TMS ethers (Table 2) and triglycerides (10).

† Single determination.

TABLE 2 RELATIVE ELUTION TEMPERATURES (T_{RE})* OF TMS ETHERS OF SATURATED DIGLYCERIDES C_4–C_{36} ON QF-1 AND SE-30

Diglyceride (Carbon Number)	Corrected Program Rate (°C/min)					
	1.85		3.71		5.56	
	QF-1	SE-30	QF-1	SE-30	QF-1	SE-30
4	0.466 ± 0.005	0.442 ± 0.006	0.455 ± 0.010	0.465 ± 0.005	0.416 ± 0.006	0.482 ± 0.020
8	0.521 ± 0.006	0.543 ± 0.005	0.532 ± 0.011	0.555 ± 0.005	0.485 ± 0.009	0.569 ± 0.009
12	0.612 ± 0.009	0.646 ± 0.004	0.637 ± 0.010	0.642 ± 0.004	0.608 ± 0.005	0.655 ± 0.007
16	0.692 ± 0.006	0.710 ± 0.002	0.711 ± 0.001	0.704 ± 0.002	0.689 ± 0.002	0.711 ± 0.002
20	0.775 ± 0.002	0.803 ± 0.000	0.794 ± 0.005	0.793 ± 0.003	0.771 ± 0.002	0.790 ± 0.003
24	0.846 ± 0.004	0.875 ± 0.002	0.864 ± 0.009	0.860 ± 0.004	0.836 ± 0.005	0.860 ± 0.003
28	0.897 ± 0.004	0.927 ± 0.002	0.917 ± 0.010	0.914 ± 0.007	0.884 ± 0.004	0.912 ± 0.003
32	0.950 ± 0.006	0.981 ± 0.004	0.970 ± 0.010	0.968 ± 0.007	0.935 ± 0.005	0.966 ± 0.000
34	0.977 ± 0.004	1.01 ± 0.00	0.993 ± 0.008	0.994 ± 0.006	0.960 ± 0.004	0.989 ± 0.003
36	0.998 ± 0.003	1.04 ± 0.01	1.02 ± 0.01	1.02 ± 0.01	0.983 ± 0.004	1.01 ± 0.00
Tri-C_8	0.813 ± 0.005	0.796 ± 0.004	0.826 ± 0.002	0.802 ± 0.004	0.825 ± 0.001	0.811 ± 0.004

Results were duplicate with absolute deviations given except for SE-30 at 5.56°C/min and QF-1 at 3.71 or 5.56°C/min where results were triplicate with standard deviations given. Sample composition as given in Fig. 1, with 2.5–12.5 μl injected.

* Glycerol trilaurate has been assigned a T_{RE} of 1.00 for direct comparison of results with those obtained with triglycerides (10) and monoglyceride TMS ethers (Table 1).

DISCUSSION

Silylation

Although other workers (4, 7, 8) have presented indirect evidence for completeness of silylation, no direct evidence is available. Despite the fact that bis(trimethylsilyl)-acetamide is a powerful TMS group donor (13), our use of thin-layer chromatography has shown that a reaction time of 1 min (8) may not be sufficient for silylation of 1,2-diglycerides and is insufficient for 1,3-diglycerides. A reaction time of at least 5 min with this reagent is therefore recommended. The extraneous peaks we observed in the triglyceride region when free or partially silylated glycerides were analyzed could be masked in the GLC analysis (7, 8) of the products of partial lipolysis of triglycerides.

Qualitative GLC Characteristics

Isothermal. The deviation of triglycerides (10) and TMS ethers of mono- and diglycerides of low molecular weights

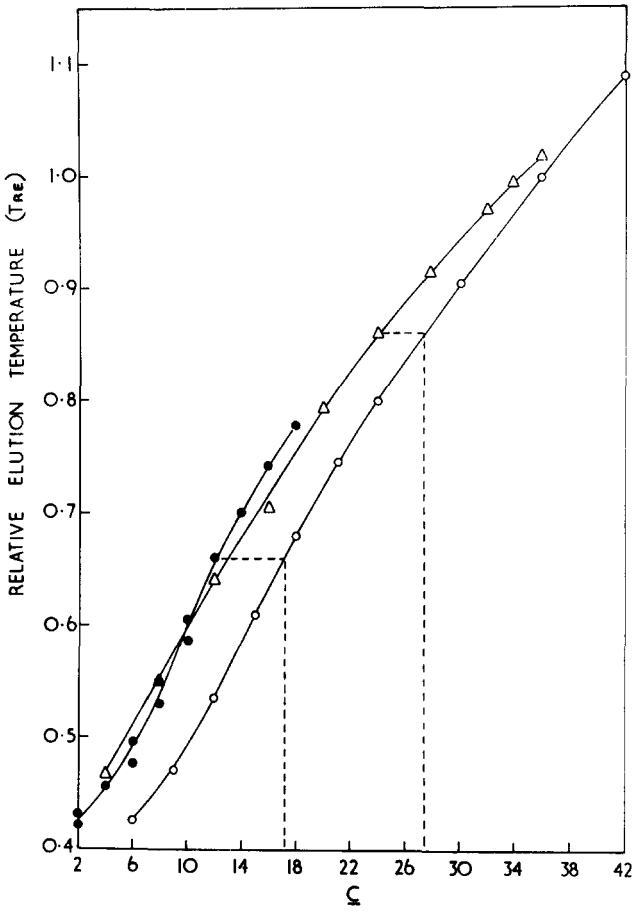

FIG. 3. Graphical determination of ΔC_{TMS} values, using the results for TMS ethers of glycerol mono- and diglycerides as examples. The TMS ethers were chromatographed on SE-30, temperature programmed at 3.71°C/min. Values of T_{RE} for TMS ethers of mono- and diglycerides are from Tables 1 and 2, respectively, and for triglycerides, from reference 10. Individual values of T_{RE} for isomers of monoglyceride TMS ethers are given. ●, TMS ethers of monoglycerides; △, TMS ethers of diglycerides; ○, triglycerides.

The TMS ether of glycerol monolaurate (C_{12}) has the same T_{RE} as "triglyceride" $C_{17.1}$. Hence the difference in carbon number is 5.1. Since there are two TMS groups attached to glycerol monolaurate, $\Delta C_{TMS} = 2.55$.

The TMS ether of glycerol dilaurate (C_{24}) has the same T_{RE} as "triglyceride" $C_{27.2}$. Hence the difference in carbon number is 3.2. Since there is only one TMS group, $\Delta C_{TMS} = 3.2$. Values of ΔC_{OH} for free diglycerides are derived in a similar manner.

(Fig. 1) from the relationship log $V_r = kC$ was more pronounced on QF-1 than on SE-30, as is to be predicted on the basis of adjoining group interaction. In view of the difference in structure of these lipids, the similarity of the deviation was unexpected. Thus, factors such as adsorption on column walls and (or) the support probably also contribute to this deviation. The need to apply several samples of the mixture of monoglyceride TMS ethers before reproducible V_r values can be obtained also speaks for this interpretation.

The modifying influence of the TMS group on V_r was clearly seen with monoglyceride TMS ethers (Fig. 1).

TABLE 3 CARBON EQUIVALENT OF A TMS GROUP (ΔC_{TMS}) FOR SATURATED MONO- AND DIGLYCERIDE TMS ETHERS ON SE-30, TEMPERATURE PROGRAMMED AT 3.71°C/MIN, AND ON QF-1, TEMPERATURE PROGRAMMED AT 5.56°C/MIN

Glyceride TMS Ether (Carbon Number)	ΔC_{TMS}	
	SE-30	QF-1
Monoglycerides		
2	1.75, 2.10*	0.25, 0.60
4	2.10	0.80
6	1.70, 2.10	1.35, 1.80
8	1.90, 2.30	2.05, 2.20
10	2.00, 2.40	2.25
12	2.55	2.00, 2.10
14	2.35	2.00, 2.10
16	2.30	2.45
18	2.30	2.30
Diglycerides		
4	4.8	—
8	4.8	−2.0
12	4.3	0.5
16	2.9	0.0
20	3.4	0.4
24	3.2	0.3
28	3.2	−0.6
32	2.0	−0.2
34	1.7	−1.4
36	1.5	−1.4

ΔC_{TMS} is the difference per TMS group in carbon number between a mono- or diglyceride TMS ether and the triglyceride with the same T_{RE} under the same conditions of temperature programming. ΔC_{TMS} may be readily found by plotting the triglyceride T_{RE} values against carbon number, and fitting the mono- or diglyceride TMS ether T_{RE} values (Tables 1 and 2) to the plot.

* Where two values of ΔC_{TMS} are given, the smaller value refers to a 1-isomer.

TABLE 4 RELATIVE ELUTION TEMPERATURES (T_{RE})* AND THE CARBON EQUIVALENT OF THE HYDROXYL GROUP (ΔC_{OH})† FOR SOME FREE DIGLYCERIDES ON SE-30, TEMPERATURE PROGRAMMED AT 5.56°C/MIN

Diglyceride (Carbon Number)	T_{RE}	ΔC_{OH}
16	0.736 ± 0.003‡	4.1
20	0.818 ± 0.008‡	4.4
24	0.874	3.5
28	0.932	3.1

* Relative to glycerol trilaurate (C_{36}).

† ΔC_{OH} is the difference in carbon number between the free diglyceride and the triglyceride with the same T_{RE} under the same conditions of temperature programming.

‡ Duplicate determinations with absolute deviations given.

Whereas triglycerides had smaller V_r values on QF-1 than on SE-30 at most temperatures (10), the reverse was true for monoglyceride TMS ethers. The effect of the TMS group on diglyceride characteristics was, as expected, less marked.

Almost equal slopes of log V_r versus $1/T$ were observed for triglycerides (10), which implies equal heats of solu-

TABLE 5 WEIGHT CORRECTION FACTORS (f_w) FOR SATURATED MONOGLYCERIDES (ANALYZED AS TMS ETHERS) ON SE-30 AT VARIOUS LOADINGS, RELATIVE TO GLYCEROL TRILAURATE (1.00)

Monoglyceride TMS Ether (Carbon Number)	Total Load (μg)*			
	211.8	127.1	84.7	42.9
2	1.46 ± 0.05	1.38 ± 0.04	1.21 ± 0.02	1.63 ± 0.06
4	0.95 ± 0.02	0.97 ± 0.02	1.18 ± 0.04	1.52 ± 0.05
6	0.92 ± 0.02	0.96 ± 0.02	0.86 ± 0.03	0.95 ± 0.02
8	0.84 ± 0.01	0.82 ± 0.02	0.80 ± 0.02	0.82 ± 0.02
10	1.00 ± 0.03	0.99 ± 0.03	0.95 ± 0.05	0.96 ± 0.05
12	0.77 ± 0.04	0.79 ± 0.02	0.86 ± 0.03	0.69 ± 0.05
14	1.07 ± 0.01	1.13 ± 0.03	1.60 ± 0.08	1.39 ± 0.03
16	0.85 ± 0.02	0.80 ± 0.01	0.91 ± 0.03	0.88 ± 0.05
18	0.84 ± 0.04	0.90 ± 0.02	0.74 ± 0.04	0.78 ± 0.05

* Percentage composition is given in Fig. 1. Column conditions were: 10% SE-30, temperature programmed from 120 to 325°C at 5.56°C/min. Glycerol trioctanoate was used as the standard for internal normalization and glycerol trilaurate (C_{36}) assigned an f_w of unity for direct comparison with f_w values of triglycerides (10) and diglycerides (Table 6). The mean of duplicate values with absolute variations are quoted.

TABLE 6 WEIGHT CORRECTION FACTORS (f_w) FOR SATURATED DIGLYCERIDES (ANALYZED AS TMS ETHERS) ON SE-30 AT VARIOUS LOADINGS, RELATIVE TO GLYCEROL TRILAURATE (1.00)

Diglyceride TMS Ether (Carbon Number)	Total Load (μg)*			
	267.7	214.2	107.1	53.8
4	5.99 ± 0.01	5.55 ± 0.09	6.18 ± 0.15	5.24 ± 0.14
8	2.81 ± 0.02	2.33 ± 0.08	2.76 ± 0.03	2.24 ± 0.05
12	3.73 ± 0.06	3.36 ± 0.09	3.80 ± 0.05	3.21 ± 0.09
16	1.75 ± 0.02	1.63 ± 0.05	1.88 ± 0.03	1.56 ± 0.02
20	2.06 ± 0.03	2.15 ± 0.02	2.32 ± 0.03	2.08 ± 0.08
24	3.12 ± 0.01	3.11 ± 0.13	3.22 ± 0.05	2.61 ± 0.05
28	2.47 ± 0.01	2.51 ± 0.06	2.93 ± 0.06	2.23 ± 0.01
32	2.04 ± 0.01	2.21 ± 0.01	2.49 ± 0.07	2.15 ± 0.04
34	2.09 ± 0.01	2.05 ± 0.02	2.05 ± 0.05	1.82 ± 0.12
36	2.28 ± 0.02	1.97 ± 0.03	1.93 ± 0.04	1.83 ± 0.05

* Percentage composition is given in Table 2. Column conditions were 10% SE-30, temperature programmed at 3.71°C/min from 200°C. Glycerol trioctanoate was used as the standard for internal normalization and glycerol trilaurate (C_{36}) assigned an f_w of unity for direct comparison with f_w values of triglycerides (10) and monoglycerides (Table 5). The mean of duplicate values with absolute variations are quoted.

tion in QF-1 and SE-30. This was not the case with mono- and diglyceride TMS ethers (Fig. 2), where larger negative apparent heats of solution in QF-1 compared with SE-30 must exist. Since adsorption on column walls and (or) the support is probable, these apparent heats of solution could be the sum of true heats of solution and heats of adsorption.

As was true for triglycerides (10), the number of theoretical plates calculated was small for mono- and diglyceride TMS ethers, though separation in terms of carbon number was adequate for our purposes.

Temperature-Programmed. Values of T_{RE} (Tables 1 and 2) showed very good reproducibility for duplicate or triplicate determinations at different rates of temperature programming. Thus the relative temperature concept of Schmit and Wynne (14) proved to be the measurement of choice, as it did for triglycerides.

The effect of the TMS group on values of T_{RE} was not exactly the same for all glycerides (Table 3). However, for the monoglyceride TMS ethers C_6–C_{18} there was little difference in the range of values of ΔC_{TMS}. Thus when temperature programming was used, the resultant

TABLE 7 COMPOSITION OF DIGLYCERIDES OBTAINED FROM EGG AND BOVINE BRAIN LECITHINS (MOLES %) AS DETERMINED BY GLC ON SE-30

Diglyceride (Carbon Number)	Moles % Derived From	
	Egg Lecithin	Bovine Brain Lecithin
30	—	1.3
32	2.1	10.6
34	79.2	68.7
36	18.6	19.2
Average fatty acid carbon number*	17.2	17.1

* Obtained from the formula: $C_a = 1/100 \times \Sigma_{30}^{36} (C/2 \times \text{mole } \%)$ where C_a = average fatty acid carbon number; C = diglyceride carbon number.

effect of the TMS group was similar for SE-30 and QF-1, unlike the effects observed isothermally. Although addition of one TMS group to a molecule means addition of the equivalent of 5.3 carbon atoms, only about two of these registered as a molecular weight increase with monoglycerides (Table 3).

The ΔC_{TMS} of 4.8 for diglyceride TMS ethers C_4 and C_8 on SE-30 implies that these ethers had characteristics similar to a triglyceride of the same molecular weight. However, on both QF-1 and SE-30, ΔC_{TMS} tended to decrease with increasing molecular weight, which indicates increasing influence of the TMS group on specific interactions and (or) adsorption. This implies that it becomes increasingly difficult to separate diglyceride TMS ethers and triglycerides of the same carbon number with increasing molecular weight.

Since values of ΔC_{TMS} (Table 3) and ΔC_{OH} (Table 4) for diglyceride TMS ethers on SE-30 were similar (i.e. free and silylated diglycerides were retained for about the same length of time), the usefulness of silylation of diglycerides lies in minimizing the adsorption on column walls and (or) the support.

Quantitative GLC Characteristics

From Tables 5 and 6, it was seen that plots of f_w versus C for both mono- and diglyceride TMS ethers were not linear, though linearity would be expected from their percentage of combustible carbon (15, 16). Neither was it possible to fit smooth curves to plots of f_w vs. C. Hence adsorption of a portion of the mono- or diglyceride TMS ether (see above) rather than carbon percentage is the predominant factor determining detector response. This adsorption effect was also seen in the influence of column load on f_w values (Tables 5 and 6), as had been noted previously for short-chain triglycerides (10).

Though their data showed that f_w values varied from determination to determination, Sahasrabuhde and Legari (9) claimed 2% reproducibility in the determination of diglycerides C_{24}–C_{32}. To obtain this degree of reproducibility, it is essential that f_w factors be checked regularly to detect changes in adsorption during the working life of a column and especially during its initial conditioning. As pointed out for triglycerides (17), the quantities of mono- or diglyceride TMS ethers applied to the column for analysis must be similar to those used for the determination of f_w.

Application to Natural Samples

Although the standard mixtures used to determine f_w values for TMS ethers of diglycerides C_{30}–C_{36} were not entirely representative of those derived from the natural lecithins analyzed, they appeared to be adequate. The values of C_a, the average fatty acid chain length (Table 7), obtained for diglycerides derived from egg and bovine brain lecithins agreed closely with values (17.22 and 17.15 for egg and bovine brain lecithins, respectively) calculated by the authors from fatty acid data presented by other workers (18, 19).

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