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Journal of Chromatography A, 967 (2002) 269–278

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Silver ion liquid-chromatographic mobility of plant diacylglycerols as a function of their composition and spatial arrangement

Andrei G. Vereshchagin*, Vasily P. Pchelkin

Laboratory of Lipid Metabolism, Institute of Plant Physiology, Russian Academy of Sciences, Botanicheskaya ul. 35, Moscow 127276, Russia

Received 15 November 2001; received in revised form 27 May 2002; accepted 31 May 2002

Abstract

Coordination complexes of unsaturated *rac*-1,2-diacylglycerols (DAGs) with silver ions were separated by adsorption and reversed-phase TLC (silver ion TLC and silver ion RP-TLC, respectively). During silver ion TLC, silver ion complexes are formed by an indeterminate number of coordination centers of various nature and only at the adsorbent surface; separation of the complexes proceeds according to an adsorption mechanism, and there is an inverse exponential relationship between DAG unsaturation and their mobility. With silver ion RP-TLC, the complexes are formed only with double bonds, only in solution, and at a 1:1 ratio; the complexes are fractionated by lipophilic partitioning between two liquid phases, and the relationship between the unsaturation of DAGs and their mobility is a direct linear one. Nevertheless, in spite of all these differences, the use of both methods demonstrated that DAG species characterized by a coiled acyl configuration always greatly exceeded in polarity those with the same unsaturation, but with the configuration close to an extended one; in the former group, this excess amounted to two- to three-fold and 30–40% for silver ion TLC and silver ion RP-TLC, respectively. In addition, for both versions of silver ion LC, these two groups of species differ from each other quantitatively, but not qualitatively, in the pattern of the relationship between the unsaturation and mobility of DAG complexes. Thus, under all conditions of silver ion LC studied here, the polarity of DAG complexes and, therefore, their mobility are conditional not only on the number of double bonds, but also on their configuration.

Keywords: Quantitative structure–retention relationships; Diacylglycerols; Silver ions

1. Introduction

In modern liquid chromatography (LC), numerous studies have been devoted to the determination of the quantitative functional relationship between the molecular descriptors of natural compounds and their chromatographic behaviour (quantitative structure–chromatographic retention relationship). The results of these studies are fundamental for understanding the physico-chemical mechanism of the interaction of the compounds under investigation with both phases of a LC system. From a practical point of view, they are essential for optimizing the conditions of LC fractionation and for an accurate prediction of the mobility of these compounds aimed at their putative identification. These results were reviewed in the widely known treatise by Kaliszan [1].

In particular, a considerable number of LC studies have been devoted to the separation of olefinic

*Corresponding author. Tel.: +7-095-903-9351; fax: +7-095-977-8018.

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00752-5

compounds, such as unsaturated aliphatic lipids, as their labile coordination complexes with silver ions; for the sake of brevity, they are designated below as ''complexes'' [2]. Studies on the relationship between the composition of fatty acid methyl ester (FAME) and triacylglycerol (TAGs) complexes and their adsorption silver ion HPLC mobility showed that an increase in the double bond number of lipids (*e*) resulted in a decline in their mobility; however, for reasons still unknown, the retention of the FAME and TAG complexes in a column was not proportional to the value of *e*, but increased much more strongly. In other words, the relationship between *e* and mobility deviated drastically from an additive one [3–8].

The complexes of FAMEs and TAGs having the same *e*, i.e. those with $\Delta e = 0$, can be, in many instances, efficiently separated from each other [6]; in particular, there was the reported separation of *cis*-monoenoic C_{18}-FAME molecular species characterized by a different double bond position in the FA chain [4]. Therefore, in these cases, the mobility of the complexes depended on some, still unknown, molecular descriptors other than *e*, possibly on the FA chain configuration. On the other hand, during the separation of silver ion complexes of unsaturated FA derivatives using reversed-phase HPLC (silver ion RP-HPLC), no effect of these molecular descriptors on their mobility was observed, and their chromatographic behaviour was determined only by the degree of unsaturation of the FA chain [5].

It can be seen that an accurate identification of unsaturated lipid molecular descriptors other than *e*, which are also responsible for differences in the LC mobility of lipid complexes, is necessary. It would be important for elucidating the, still poorly understood, mechanisms of interaction between these compounds and Ag^+, as well as between the complexes thus formed and the separate phases of LC systems [6]. Earlier, we performed a series of thin-layer chromatographic (TLC) studies on separating a mixture of unsaturated 1,2-diacyl-*rac*-glycerols (DAGs), using RP-TLC in the absence of Ag^+ [9], silver ion adsorption TLC (referred to below as ''silver ion TLC'' [10]), and silver ion RP-TLC [11], into individual molecular species. The objective of the present work was to employ the results of these studies as a possible contribution to solving the problem outlined above. We show that the spatial configuration of DAG hydrocarbon chains is one of the molecular descriptors affecting the mobility and, hence, the polarity of DAG complexes.

2. Experimental

The experimental techniques used in this work were described in detail previously [9–11]. Briefly, a model preparation of plant DAGs used as the subject of the study was obtained using a TAG mixture from cocoa, poppy seed, and linseed oils. This mixture included 20 mol% each of palmitic (P), stearic (St), oleic (O), linoleic (L), and linolenic (Le) acid residues. The mixture was subjected to catalytic transesterification of TAGs with glycerol [9]. The DAGs obtained were isolated from other products of this reaction using column adsorption chromatography and TLC on a silica gel/boric acid plate [10].

In the first series of experiments, unsaturated DAG complexes were separated by continuous-flow silver ion TLC using a chloroform–isopropanol (99:1, v/v) mixture as mobile phase [10]. Prior to separation, the silica gel plate with a specific surface area of about 400 m^2/g was impregnated with a 1% (w/v) methanolic solution of silver nitrate. As a result, the silver content throughout the plate was uniform and amounted to about 0.1 mg Ag/m^2 silica specific surface area. TLC zones of DAGs were detected by treating the plate with a 5% aqueous–alcoholic solution of phosphomolybdic acid followed by heating at 120 °C for 10 min. The mobility of these zones (R_1) was determined in relation to the mobility of a diacylglycerol external standard, 1,3-LL [10]. The latter was chosen as the standard because it was absent from the model DAG preparation, and its TLC zone was well separated from those of all components of this preparation [10].

In the second series of experiments, the DAG complexes were separated by continuous-flow silver ion RP-TLC. A plate with the same silver content (see above) was impregnated with a 10% (v/v) benzene solution of *n*-tetradecane, and a 5% methanolic solution of boric acid saturated with silver nitrate and; *n*-tetradecane was used as mobile phase. DAG zones were detected as described above, and their mobility (R_2) was determined in relation to that of *rac*-1,2-LeLe; the latter was present in the model DAG preparation and exhibited the highest silver ion

RP-TLC mobility compared with other molecular species of the preparation [11].

Individual DAG species in the model preparation were previously identified according to their FA composition by comparing them with standard DAG mixtures as regards the qualitative and quantitative composition of separate TLC zones and the relative mobility of the latter [10]. For DAG identification, the composition of the model preparation was also compared with a random one, viz. the DAG composition calculated on the basis of the theory of the random distribution of FA residues between DAG molecules. Such a comparison was justified, because this preparation was obtained by catalytic TAG transesterification with glycerol [9]. The fact that this process results in a random distribution of FAs in the DAGs thus obtained was first established by Brandner and Birkheimer [12].

3. Results and discussion

3.1. Silver ion TLC

The relationship between the mobility of DAG molecular complexes and the e value on silver ion LC was first investigated using the results of silver ion TLC [10]. As shown in Table 1 (column 2), the species differing in e by 1 or more, i.e. the species with $\Delta e \geq 1$, are always separated from each other, whereas some components differing only in the nature of the saturated (S) fatty acyl (S = St or P), i.e. those with $\Delta e = 0$ (StL and PL; StLe and PLe), coincided in their R_1 value. The members of the other five pairs of species with $\Delta e = 0$, viz. StL and OO with $e = 2$, PL and OO with $e = 2$, StLe and OL with $e = 3$, PLe and OL with $e = 3$, and OLe and LL with $e = 4$, were efficiently separated from each other. Therefore, their R_1 value depended not only on the value of e, but also on some other DAG molecular descriptor(s).

In the search for a reason for this phenomenon, we turned our attention to the fact that, for each of the above DAG pairs, the first component differs from the second in having a lower R_1 value, i.e. a higher polarity of its complex. Therefore, in our DAG preparation, we could distinguish two groups of species: a more polar group (StL, PL, StLe, PLe, and OLe; Group I) and a less polar group at $\Delta e = 0$ (OO, OL, and LL; Group II).

Table 1
Functional relationships between the degree of unsaturation (e) of diacylglycerols and their relative mobility during silver ion TLC (R_1) and silver ion RP-TLC (R_2)

DAG species (e)	Silver ion TLC				Silver ion RP-TLC			l[b]	L[c]
	Found $R_1 \pm s$[a]	R_1, calculated by		p	Found $R_2 \pm s$[a]	R_2, calculated by			
		Eq. (1)	Eq. (2)			Eq. (12)	Eq. (13)		
1	2	3	4	5	6	7	8	9	10
StO (1)	4.88±0.07	–	4.88	1.03	0.15±0.01	–	0.13	33	33
OO (2)	2.50±0.06	–	2.42	2.06	0.30±0.02	–	0.27	30	30
StL (2)	1.47±0.09	1.47	–	2.46	0.30±0.02	0.33	–	31	30
PL (2)	1.47±0.09	1.47	–	2.46	0.38±0.03	0.33	–	29	28
OL (3)	0.94±0.06	–	1.20	3.49	0.43±0.03	–	0.41	28	27
LL (4)	0.60±0.04	–	0.60	4.92	0.51±0.02	–	0.54	29	24
StLe (3)	0.33±0.03	0.44	–	5.45	0.51±0.02	0.50	–	26	24
PLe (3)	0.33±0.03	0.44	–	5.45	0.60±0.02	0.50	–	27	22
OLe (4)	0.20±0.03	0.13	–	6.48	0.69±0.03	0.67	–	26	21
LLe (5)	0.10±0.02	0.04	–	7.91	0.83±0.03	0.83	–	24	18
LeLe (6)	0.03±0.01	0.01	–	10.90	1	1.00	–	22	12
DAG group		I	II	–	–	I	II		
r[d]		0.999	0.997	–	–	0.986	0.994		

[a] s, absolute standard deviation of individual R_1 and R_2 measurements.
[b] $l = m - 2e - u$, uncorrected lipophilicity of DAG complexes.
[c] $L = m - 2p - u$, corrected lipophilicity of DAG complexes.
[d] r, regression coefficients between the found R_1 and R_2 values and those calculated according to Eqs. (1) and (2) and Eqs. (12) and (13).

It is suggested that the distinction between Group I and Group II DAGs in their polarity may be caused by the different spatial configurations of their component FA residues. As reported by Brenner [13], the aliphatic chain configuration in S and O acids is close to an extended one, whereas the introduction of two or more *cis* double bonds is known to induce profound configurational changes in the chain and the shortening of the latter. Thus, for St, O, L, and Le acids at 25 °C, the average chain length is 1.40, 1.20, 1.13, and 1.02 nm, respectively (see Fig. 10 of Ref. [13]). As a result, a polyunsaturated hydrocarbon chain acquires a highly coiled and more rigid configuration. As an example, the configuration of O and L acids is given in Fig. 1.

It can also be seen that, in each Group I DAG species, the FA residues are drastically different from one another in their spatial configuration. In contrast, in Group II, the OO and LL species contain identical FAs, and the OL species contains FAs that are somewhat similar to each other in structure. Thus, we believe that, in Groups I and II, the R_1 of complexes depends not only on *e*, but also on the configurational characteristics of the DAG and it is

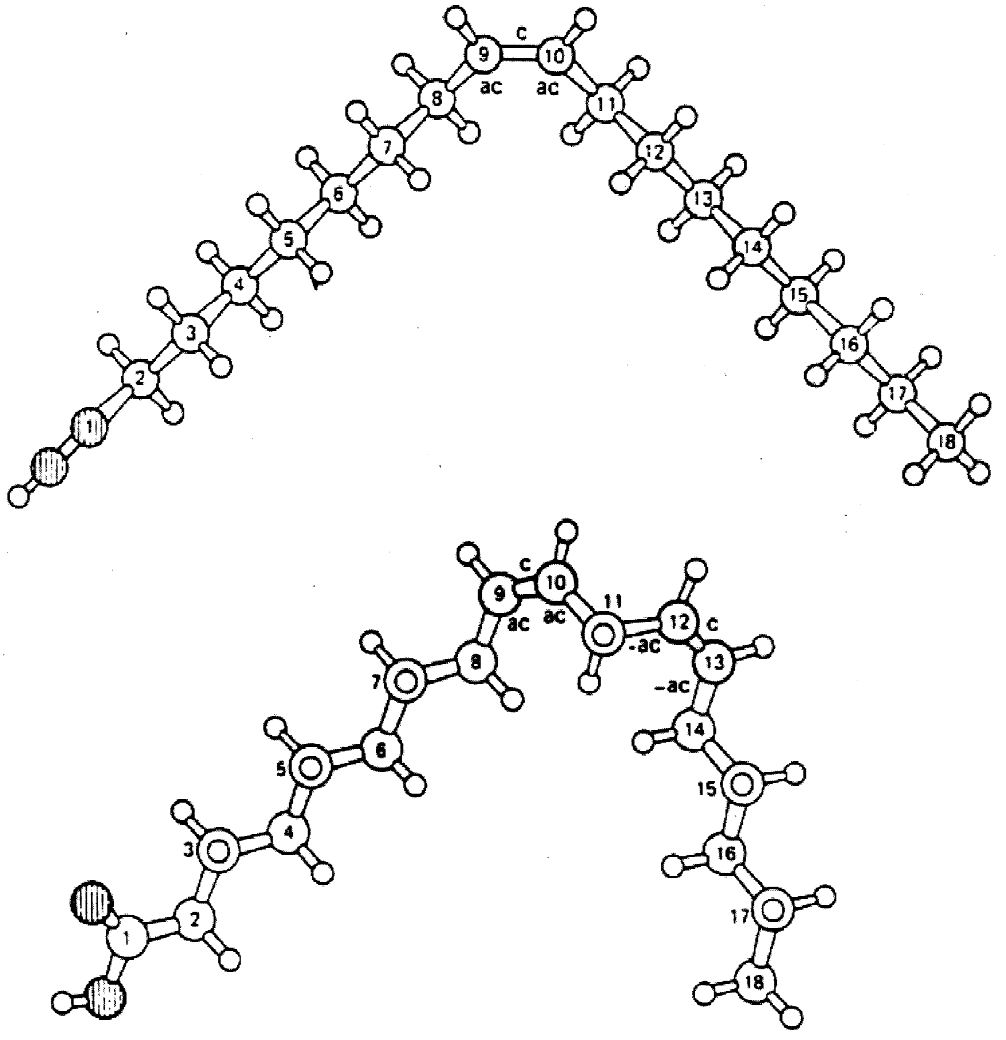

Fig. 1. Configuration of oleic and (bottom) linoleic acids. c, *cis* double bond; ac, anti-clinal rotametric structure of C–C bonds. From Brenner [13].

this molecular descriptor that causes the differences between the groups in the R_1 values of DAGs with $\Delta e = 0$.

To establish a functional relationship between R_1 and quantitative molecular descriptor(s) of unsaturated DAGs, we adopted the *e* value as the descriptor to be studied. Comparison of the *e* and R_1 values in Table 1 (columns 1 and 2) shows that the transition from StO to LeLe is accompanied by an increase in *e* of less than an order of magnitude; the R_1 values decrease by more than two orders. Therefore, it is expected that the *e* vs. R_1 relationship is exponential. To quantitatively assess the possible contribution of the configurational characteristics of individual DAG species to this relationship, the latter was determined separately for Groups I and II (Table 1, columns 3 and 4) rather than for the totality of DAGs (column 1). For this determination, the LLe and LeLe species, which contain highly coiled chains of Le residues, were additionally included in Group I. The StO species, which, like OO, is characterized by a configuration close to an extended one, was included in Group II. Thus, for the determination of the *e* vs. R_1 functional relationship, Group I consisted of StL, PL, StLe, PLe, OLe, LLe, and LeLe, while Group II included StO, OO, OL, and LL.

The calculation using the *e* and R_1 values from Table 1 (columns 1 and 2) showed that, for Group I, this relationship can be described by

$$R_1 = 4.88 \exp(1.2 - 1.2e) \quad (1)$$

and, for Group II, by

$$R_1 = 4.88 \exp(0.7 - 0.7e) \quad (2)$$

Thus, for both groups, the *e* vs. R_1 functional relationship is qualitatively exponential. These groups differ markedly from each other in the quantitative characteristics of this relationship, and the distinction between them with respect to the numbers at *e* reflects a decreased R_1, i.e. an increased polarity of the complexes of Group I DAG species compared with the respective Group II species.

The actual existence of Groups I and II in the model DAG preparation is also demonstrated by the fact that the calculation of the *e* vs. R_1 relationship for *the totality* of unsaturated DAG species (Table 1, column 1) according to Eqs. (1) and (2) yields, respectively, regression coefficients of $r = 0.970$ and 0.958, which are considerably lower than those calculated for each group separately ($r = 0.999$ and 0.997). In the latter instance, the calculated R_1 values (Table 1, columns 3 and 4) are, as a whole, fairly consistent with the determined values (column 2).

It is of interest to compare our data for the silver ion TLC of DAG complexes [Table 1, columns 1–4; Eqs. (1) and (2)] with the results obtained by Nicolova-Damyanova et al. [8]. These authors determined the retention properties (*k*) of 19 unsaturated TAG species ($e = 1$–9) from common vegetable oils on adsorption silver ion HPLC. For this comparison, the ratios of the *k* value of a TAG species to the *k* value of a standard SSO species (k_{SSO}) were used to calculate the TAG relative mobility (R_{SSO}) values. To perform this calculation, the equation

$$R_f = 1/(k + 1) \quad (3)$$

where R_f is the relative TLC mobility of a particular TAG species [14], was expressed in terms of the equations

$$d_{TAG}/d_f = 1/(k_{TAG} + 1) \quad (4)$$

and

$$d_{SSO}/d_f = 1/(k_{SSO} + 1) \quad (5)$$

where d_{TAG}, d_{SSO}, and d_f are the virtual distances covered by the chromatographic zone of a particular TAG species, the zone of the SSO standard, and the mobile phase front *f*, respectively, from the starting point of the chromatogram, and k_{TAG} is the *k* value of a particular TAG species. By dividing the left- and right-hand sides of Eq. (4) by the respective sides of Eq. (5), we obtain

$$d_{TAG}/d_{SSO} = R_{SSO} = (k_{SSO} + 1)/(k_{TAG} + 1) \quad (6)$$

Comparison of the R_{SSO} values for individual TAG species, calculated from the *k* data [8], with their *e* values showed that the *e* vs. R_1 functional relationship is exponential:

$$R_{SSO} = \exp(2.3 - 2.3e), \; r = 0.997 \quad (7)$$

Thus, the results of Ref. [8] are consistent with our

conclusion on the existence of an exponential relationship between e and R_1 for DAG complexes.

These results support our inference about the effect of the configurational characteristics of the complexes on the value of R_1. First, this concerns the disproportionally reduced silver ion LC mobility of Le-containing lipid complexes. Thus, the ratio between SLe ($e = 3$), OL ($e = 3$), and SO ($e = 1$) with regards to their R_1 values is 1:3:15 (Table 1), while, for silver ion HPLC of TAG complexes, the respective ratio between the R_{SSO} values of SSLe ($e = 3$), OOO ($e = 3$), and SSO ($e = 1$) is 1:6:109 [8].

This sharp distinction may be a result of the fact that TAGs, as compared to DAGs, are characterized by a more rigid structure, where the FA chains are more tightly attached to the glycerol backbone [8]. This suggestion is confirmed, first of all, by the results for silver ion HPLC of FAME complexes, in which the chains are not bound to any rigid skeleton. In this case, the ratio between the esters of Le ($e = 3$), L ($e = 2$), and O ($e = 1$) with regard to the R_1 value for their complexes calculated according to Eq. (3) is 1:2.4:4.5 [4]; thus, a three-fold increase in e is accompanied by only a 4.5-fold decrease in the ratio rather than by 15- and 109-fold as with DAGs and TAGs. Moreover, our suggestion is confirmed by comparing Eq. (7) for TAGs, Eq. (1) for DAGs, and the equation analogous to Eq. (7) for FAMEs with regard to the numbers at e, which are 2.3, 1.2, and 1.0, respectively. Thus, the dependence of the mobility of the complexes on the configuration of the lipid molecules manifests itself not only in Groups I and II of DAGs (Table 1), but also in other classes of neutral lipids.

The importance of the shape of the DAG molecules in the adsorption-chromatographic behaviour of their complexes was also demonstrated by Itabashi et al. [15]. These authors separated the 3,5-dinitrophenylurethane derivatives of 1,2-diacyl-*sn*-glycerols by silver ion HPLC using a silver-loaded Chromsphere 5 Lipids cation-exchange column at 10 °C with chloroform–acetonitrile (200:1, v/v) as the mobile phase at a constant flow-rate of 0.5 ml/min. Under these conditions, the complex of 1-palmitoyl-2-oleoyl-*sn*-glycerol, the sterically more hindered isomer having an unsaturated acyl chain in the secondary position, was eluted faster than its reverse isomer with an unsaturated chain in the primary position, viz. the complex of 1-oleoyl-2-palmitoyl-*sn*-glycerol (cf. Fig. 9A of Ref. [15]). These results support the conclusions of the present work.

Moreover, it should be noted that the differences between the S and O acids, on the one hand, and the polyunsaturated acids, on the other, in the configuration of their aliphatic chains not only determine the polarity of DAG complexes containing these FAs, but also the behaviour of these FAs during their fractionation by urea crystallisation. Thus, due to the selective sedimentation of urea inclusion compounds of S and O, the content of L in a total FA mixture obtained from safflower seed oil increased from 76 to 97% [16]. In a similar experiment using FAs from borage seed oil, the total concentration of linoleic and γ-linolenic acids increased from 61 to 99% [17].

The physico-chemical mechanism resulting in an inverse exponential pattern of the relationship between e and the mobility values of unsaturated lipid complexes during adsorption silver ion LC has not been established thus far [4]. One can only suggest that this mechanism involves, to some extent, a recently reported reversible interaction of a single silver ion bound by the silanol groups of the adsorbent surface with two lipid coordination centers simultaneously [4]. On the one hand, these centers are believed to be represented by two isolated olefinic bonds situated either in the same aliphatic chain or in different chains of a particular lipid. On the other hand, the two centers can consist of a double bond and a lipid ester group coordination center formed due to the free electron pair at the carbonyl oxygen atom [7,8]. Such an interaction of a silver ion with an indeterminate number of lipid coordination centers results in the formation of polar chelate complexes with charge transfer [6]. In the complexes thus formed, the polyenoic lipid chains, the active centers of which can be located at a considerable distance from each other, form pseudocyclic structures ‘‘spread’’ on the adsorbent surface. The double bonds of these adsorbed immobilized structures turn out to be located near superficial silver ions, i.e. in a position favourable for interaction with these ions. Therefore, a considerable increase in the extent of this interaction is accompanied by a decrease in the affinity of the methylene groups of these structures for the lipophilic mobile phase [3]. As a result, the residence time of unsatur-

ated DAG complexes in the stationary phase is increased, their R_1 is markedly decreased, and the *e* vs. R_1 relationship becomes exponential [8].

Finally, it should be stressed that all the above proposals are by no means established, and, to determine the true reason for the occurrence of an exponential *e* vs. R_1 relationship, further studies are necessary.

3.2. Silver ion RP-TLC

It is known that neutral-lipid complexes, including those of DAGs, can also be fractionated by the silver ion RP-TLC technique [11]. This technique was first devised as long ago as 1965 for the analysis of FAME and TAG mixtures of plant origin [2]. Later, it evolved into silver ion RP-HPLC, used for fractionating *cis*- and *trans*-isomers of Δ9-octadecenoic acid and 1,5,9-cyclododecatriene [18].

In the course of silver ion RP-TLC, as a variation of partition RP-LC [9], the mobility of the DAG complexes (R_2) can be expected to be inversely proportional to their lipophilicity. Previously, we attempted to express DAG lipophilicity (*l*) using the equation

$$l = m - 2e - u \tag{8}$$

because *l* is known to be associated with DAG molecular descriptors such as the total acyl carbon number (*m*), on the one hand, and *e* and the total number of unsaturated FA residues (*u*), on the other, by a direct and an inverse linear relationship, respectively [11].

The *l* values thus calculated are shown in Table 1 (column 9). It can be seen that there is indeed an inverse *l* vs. R_2 relationship (column 6). However, this relationship varies irregularly, and, in two instances, an increase in R_2 is even accompanied by an increase in the calculated *l* rather than by a decrease. This irregularity is suggested to be brought about by the fact that, when calculating *l*, we did not take into account the differences between the DAG species with respect to their spatial configuration. It was shown above that, with silver ion TLC, only consideration of these differences made it possible to accurately establish functional relationships between the mobility and the polarity (and, therefore, the lipophilicity) of DAG complexes.

To test this suggestion, we calculated DAG lipophilicity using the parameter *p*, an empirical value reflecting the relative adsorption capacity (i.e., relative polarity) of individual DAG complexes under the conditions of silver ion TLC [19]. The *p* value is additive:

$$p = p_1 + p_2 \tag{9}$$

where $p_1 = p_S$ or p_U and $p_2 = p_S$ or p_U are the adsorption capacities of saturated (S) or unsaturated (U) FA residues present in the *rac*-1- and *sn*-2-positions of a particular *rac*-1,2-DAG species; for individual FA species, $p_U = p_O$, p_L, or p_{Le}.

The calculation of p_1 and p_2 for each UFA species was based on the value of R_1 of the respective monoacid UU-DAG species (OO, LL, and LeLe). Because, with silver ion TLC, this value is exponentially related to *e* (see above), we assumed that the same relationship will hold for the adsorption capacity values, p_1 and p_2. Thus, p_U values were calculated using the empirical equation

$$p_U = \ln[(R_1)_{SS}/(R_1)_{UU}] \tag{10}$$

where $(R_1)_{SS} = 7.00$ [10], and $(R_1)_{UU}$ for OO, LL, and LeLe are 2.50, 0.60, and 0.03, respectively (Table 1, column 2). The calculation of p_S by Eq. (10) yields $p_S = \ln[(R_1)_{SS}/(R_1)_{SS}] = 0$. The p_O, p_L, and p_{Le} values calculated according to this equation are 1.03, 2.46, and 5.45, respectively [19].

The *p* values of unsaturated DAG complexes calculated using Eq. (9) are shown in Table 1 (column 5). It can be seen that these values, like *e*, are inversely proportional to *l* and R_2, but, in contrast to *e*, they allow for differences in the configuration of the DAG complexes. Therefore, the *p* values were used for calculating the ''corrected'' lipophilicity of DAG complexes in silver ion RP-TLC (*L*) using Eq. (11), which is a modified version of Eq. (8):

$$L = m - 2p - u \tag{11}$$

L values rounded off to the nearest whole number are shown in Table 1 (column 10). It can be seen that, in column 10, the sequences of DAG species produced by arranging the latter in the order of either

decreasing L or increasing R_2 (column 6) are completely continuous. Thus, due to the fact that the calculation of L values, in contrast to that of l (see above), took into account the contribution of the configuration to the R_2 of DAG complexes, these values reflect DAG mobility much more accurately. Correspondingly, the inverse L vs. R_2 relationship was far more tight ($r = -0.999$) than that between l and R_2 ($r = -0.972$). Therefore, in Table 1 (columns 9 and 10), the l and L values are designated, respectively, as the ''uncorrected'' and ''corrected'' DAG complex lipophilicity.

Thus, in spite of the fact that the p values were derived from the results of the *adsorption* silver ion TLC of DAG complexes, they turned out to be very useful for the quantitative characterization of R_2 of these complexes under the conditions of *reversed-phase* silver ion TLC. It can be seen that, both with silver ion TLC and silver ion RP-TLC, which differ drastically with respect to the mechanism of lipid separation (see below), the configuration of the DAG complexes markedly (and in the same direction) affects their lipophilicity (and hence their polarity).

Turning to the determination of the functional relationship between the molecular descriptors of DAG complexes and their R_2 value in silver ion RP-TLC, one must immediately emphasize that the inverse L vs. R_2 relationship cannot be considered as a strictly functional one. This limitation is caused by the fact that the calculation of L was based not only on the DAG molecular descriptors (m and u), but also on the empirical p values obtained from the results of DAG mobility, in another LC system. Therefore, as in the case of silver ion TLC, the e value was selected as an independent DAG molecular descriptor. As shown in Table 1, an increase in e from 1 to 6 (column 1) is accompanied by a 6.7-fold increase in R_2 (column 6). Thus, during silver ion RP-TLC, as in RP-TLC in the absence of silver ions [9], the e vs. R_2 relationship is close to a direct linear one.

It has been demonstrated that, to accurately estimate DAG molecular descriptors such as lipophilicity, it is necessary to allow for the parameter p reflecting the DAG configuration. Close inspection of Table 1 (columns 1 and 6) shows that, for each of the pairs of DAG species with $e = 2$, 3, 3, and 4 (PL and OO; StLe and OL; PLe and OL; OLe and LL, respectively), the first member is more mobile, i.e. more polar, than the second member. Thus, there are two DAG groups, which, at $\Delta e = 0$, are characterized by a higher (PL, StLe, PLe, OLe) and a lower (OO, OL, LL) polarity of their complexes. It is evident that these DAG groups, both in their composition and in the relationship between their polarity values, are similar, respectively, to Groups I and II found for silver ion TLC. Therefore, a direct linear e vs. R_2 relationship during silver ion RP-TLC can be determined separately for Group I:

$$R_2 = 0.167e \quad (12)$$

and for Group II:

$$R_2 = 0.135e \quad (13)$$

It can be seen that these groups differ markedly from each other in the angular coefficient of this relationship. The R_2 values calculated by Eqs. (12) and (13) and presented in Table 1 (columns 7 and 8) are, as a whole, similar to those determined (column 6). The somewhat lower r value for Group I is caused by the fact that it included DAG species with the same e (StL and PL with $e = 2$, and StLe and PLe with $e = 3$), but with different R_2 in silver ion RP-TLC.

As in the case of silver ion TLC (see above), distinguishing Groups I and II to characterize the quantitative differences in the direct linear e vs. R_2 relationship was warranted by calculating this relationship using Eqs. (12) and (13) for *the totality* of DAG species (column 1). Indeed, the r values thus obtained (0.983 and 0.966, respectively) are markedly lower than those obtained for the separate groups (columns 7 and 8). All these facts demonstrate that the configuration of the DAG species considerably affected the mobility of their complexes in silver ion RP-TLC, as was also the case in silver ion TLC.

With regard to the direct linear e vs. R_2 relationship in the silver ion RP-TLC separation of DAG complexes, it cannot, at present, be confirmed by independent evidence, because no such separation of acylglycerols has been performed. Also (see Introduction), the silver ion RP-HPLC separation data were obtained for the phenethyl and phenacyl esters of St, O, L, and Le acids [5]. Table III of Ref. [5] presents values for their retention in relation to those

for the respective myristoyl esters, and Fig. 4 of Ref. [5] shows plots of the relationship between m of saturated C_{12-18} FA esters and $\log k$ of those esters. The $\log k$ and k values for the phenethyl and phenacyl esters calculated by us from the data of Ref. [5] are shown in Table 2 (columns 2, 3, 6, and 7). Using these values and Eq. (3), we determined the relative mobility of individual phenethyl and phenacyl ester species (R_3 and R_4, columns 4 and 8, respectively). Comparison of R_3 and e (column 1) of a particular ester species shows that, for phenethyl esters, the relationship between these values can be described by the linear equation

$$R_3 = 0.15e + 0.15 \quad (14)$$

and for phenacyl esters, by the equation

$$R_4 = 0.16e + 0.16 \quad (15)$$

The close correlation between the determined R_3 and R_4 values and those calculated using Eqs. (14) and (15) is shown in columns 5 and 9, respectively. Thus, also in this case, there was a direct linear relationship between the silver ion RP-LC mobility of neutral lipids (FA aromatic esters) and their e value. As mentioned above (see Introduction), virtually no effect of the molecular descriptors of these esters, other than e, on their mobility values was evident, as the angular coefficients in Eqs. (14) and (15) and those in Eqs. (12) and (13) are almost identical. It is suggested that this difference is due to the fact that the esters studied by Nicolova-Damyanova et al. [5] included only a sole FA chain rather than two in the DAG complexes.

The data presented here make it possible to consider putative mechanisms for DAG complex formation and their interaction with separate phases of the silver ion RP-TLC system. Under these conditions, the complexes were formed only with a double bond and only in methanolic medium (see Experimental). Moreover, the complexes thus formed freely partitioned in the course of their separation between the mobile (polar) and stationary (lipophilic) phases of the TLC system, as was also the case during ordinary RP-TLC in the absence of silver ions [9]. Finally, there was no prolonged retention of complexes by the stationary phase. With m equal, DAG polarity could only be increased at the expense of silver ion binding by their olefinic bonds. Moreover, the existence of a direct linear relationship between DAG polarity and e demonstrates that, in this instance, the hydrophilic lipid complexes are formed by a reversible interaction of each double bond with only one silver ion [5].

4. Conclusion

In spite of the drastic qualitative distinction between the two versions of the TLC of DAG complexes, differences in DAG mobility are caused only by differences in their polarity. The latter results both from the various compositions of these species (primarily e) and from their configuration. In the

Table 2
Functional relationships between the degree of unsaturation (e) of fatty acid phenethyl and phenacyl esters and their relative mobility (R_3 and R_4) during silver ion RP-HPLC [5]

Acyl residue of a particular ester species (e)	Fatty acid ester							
	Phenethyl				Phenacyl			
	$\log k$ [5]	k	Found R_3	R_3, calculated by Eq. (14)	$\log k$ [5]	k	Found R_4	R_4, calculated by Eq. (15)
1	2	3	4	5	6	7	8	9
St (0)	0.85	7.0	0.12	0.15	0.55	3.5	0.22	0.16
O (1)	0.42	2.5	0.28	0.30	0.28	1.9	0.34	0.32
L (2)	0.17	1.5	0.40	0.45	0.04	1.1	0.48	0.48
Le (3)	−0.16	0.7	0.59	0.60	−0.30	0.5	0.67	0.64
r[a]				0.996				0.994

[a] r, regression coefficients between the found R_3 or R_4 values and those calculated according to Eqs. (14) and (15).

absence of silver, the structural differences between DAGs with $\Delta e = 0$ do not affect their polarity [9], but the formation of their complexes results in a clear-cut manifestation of such differences [10]. Regardless of the mode of formation, the polarity of Group I DAGs always *quantitatively* exceeds that of the respective Group II species with $\Delta e = 0$ by two- to three-fold in adsorption TLC and by 30–40% in RP-TLC of the complexes. The distinct configurational characteristics of the DAG did not affect the *qualitative* characteristics of the mobility vs. e relationship, which was inverse exponential for silver ion TLC and direct linear for silver ion RP-TLC.

Moreover, the effect of DAG structure on the polarity of their complexes, and the similarity between the two versions of TLC with respect to the properties of these complexes, are demonstrated by the fact that the most accurate assessment of the relationship between the R_2 of DAG complexes during silver ion RP-TLC and their lipophilicity (L) was achieved by determining L from an adsorption parameter, p, determined from the results of another version of silver ion LC, viz. silver ion TLC, drastically different from silver ion RP-TLC (Table 1). Finally, the effect of lipid structure on the mobility of their complexes was demonstrated during adsorption LC of TAGs and FAMEs [4,8]. It is expected that, in the future, similar results will also be obtained using silver ion RP-TLC.

Acknowledgements

This work was supported by the Russian Foundation for Basic Research, project No. 99-04-49208.

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