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## CHROMATOGRAPHIC PROPERTIES OF SOME LONG-CHAIN ALIPHATIC HALOGEN COMPOUNDS

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### SUMMARY

Long-chain halogen compounds obtained from soya bean oil and cod liver oil methyl esters by replacing the  $-\text{CO}_2\text{Me}$  groups with either  $-\text{Cl}$ ,  $-\text{Br}$  or  $-\text{I}$ , can be separated into unsaturation classes by silica gel thin-layer chromatography with alkane mobile phases. These separations may be explained by considering the interactions of the halogen atoms and the olefinic  $\pi$ -electrons with the silanol groups of a silica surface. Gas-liquid chromatographic separation factors of these halogen compounds are found to be similar to those of the corresponding methyl esters, methyl ketones and acetates.

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### INTRODUCTION

Recent biomedical interest in alkoxy glycerolipids was generated by the discovery of elevated levels of these lipids in a variety of mammalian tumours<sup>1,2</sup>, and it has subsequently been proposed that abnormally high levels of alkoxy lipids can be used as an internal marker for malignant tumours<sup>3</sup>. Alkoxy glycerolipids are also of significance in a wide range of marine organisms<sup>4</sup>. At present, in the authors' laboratory, investigations are being made on the neutral lipid composition of marine algae with emphasis on alkoxy glycerolipids.

Most methods currently available for studying the composition of the alkyl chains of alkoxy glycerolipids fall into two categories. Firstly, those which involve derivative formation of the diol function, *e.g.*, di-acetate, di-trimethylsilyl ether<sup>6</sup>, isopropylidene derivative<sup>7</sup> and examination of these derivatives by gas-liquid chromatography (GLC). However, this GLC examination is not entirely satisfactory since these derivatives have relatively long retention times and there are poor separations between the derivatives of a saturated alkoxy glycerolipid and the corresponding mono-unsaturated compound.

The second group of methods involves the cleavage of the ether bond to give, as one of the products, a long-chain alkyl halide. These halides can be used to furnish proof of the presence of alkoxy lipids<sup>8</sup>. A number of methods have been proposed

for the ether cleavage including the use of hydriodic acid<sup>9</sup>, boron trichloride-chloroform<sup>10</sup>, sodium borohydride-iodine<sup>11</sup> and very recently iodotrimethylsilane<sup>12</sup>.

Very little published chromatographic data are available for long-chain saturated and unsaturated alkyl halides and the aim of the present work is to obtain background thin-layer chromatography (TLC) and GLC data for use in subsequent structural studies of alkoxy glycerolipids.

## EXPERIMENTAL

Long-chain saturated alcohols and alcohols obtained from soya bean and cod liver oil methyl esters by reduction of these esters with aluminium lithium hydride<sup>13</sup>, were converted to the corresponding methane sulphonate<sup>14</sup>. These last compounds were then converted to long-chain halides by a Finkelstein-type reaction<sup>15</sup>: the methane sulphonates (5 mmol) were refluxed for 1 h with a solution of the appropriate sodium halide (2 M in acetone, 30 ml). The acetone mixture was then concentrated by distillation to about one half volume and water (30 ml) added.

The long-chain halides were extracted with diethyl ether and this solution dried over sodium sulphate. Traces of polar impurities were removed by passing the dried solution down a short silica gel column. The purified solutions were stored in the dark.

Long-chain acetates<sup>13</sup> and methyl ketones<sup>16</sup> were prepared as described previously.

### *Chromatography*

All solvents were distilled from glass apparatus before use.

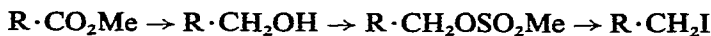
TLC was carried out on layers of silica gel (Camag, Muttenez, Switzerland; without binder) in glass tanks lined with filter paper. The layers were 0.25 and 0.75 mm for analytical and preparative separations, respectively. Analytical plates were activated at 120° for 30 min, and preparative plates at 120° for 4 h.

Halides were purified using a short glass column (6 in. × 1/4 in.) using the same silica gel as for TLC.

GLC was carried out on a PE 800 chromatograph using the following packed 6 ft. × 1/8 in. stainless-steel columns at 180° with nitrogen as the mobile phase: (1) 5% DEGS on Chromosorb W AW HMDS and (2) 5% BDS on Chromosorb W AW HMDS.

## RESULTS

In a preliminary experiment soya bean oil methyl esters were converted to iodides by the following scheme:



The iodides contained small amounts of polar impurities and a purification was carried out by passing a solution of the impure iodides down a short silica column. The effluent from the column was monitored by GLC and it was found that there was a partial separation into unsaturation classes, the saturated iodides

being concentrated in the initial fractions and the more unsaturated iodides being concentrated in later fractions. The purified soya bean oil iodides were examined by silica gel TLC with hexane as the mobile phase and a separation into unsaturation classes was obtained. When the iodides prepared from cod liver oil methyl esters were examined by TLC, six bands were obtained corresponding to the following unsaturation classes:

No. of C=C	0	1	2	3	4	5	6
$R_F$	0.61	0.51	0.42	0.34	0.28	0.24	0.21

The  $R_F$  values of seed oil chlorides, bromides and iodides on silica gel TLC with hydrocarbon mobile phases are given in Table I. With each of the mobile phases and, for each unsaturation class, the  $R_F$  values increase in the order chlorides < bromides < iodides. For each of the halides there was a decrease in  $R_F$  values with increasing density of the mobile phase. The separation factors for each pair of unsaturated halides decreased with increasing boiling point of the mobile phase, cyclohexane (b.p. 81°) giving similar separation factors to those obtained with petroleum ether (b.p. 80–100°).

TABLE I  
TLC  $R_F$  VALUES FOR LONG-CHAIN ALIPHATIC HALOGEN COMPOUNDS

Mobile phase	Density of mobile phase	No. of C=C in solute	$R_F$		
			Chlorides	Bromides	Iodides
Pentane (b.p. 36°)	0.63	0	0.64	0.65	0.73
		1	0.47	0.49	0.55
		2	0.33	0.37	0.40
		3	0.24	0.26	0.28
Light petroleum (b.p. 40–60°)	0.64	0	0.52	0.55	0.60
		1	0.40	0.42	0.47
		2	0.30	0.32	0.35
		3	0.22	0.25	0.24
Light petroleum (b.p. 80–100°)	0.70	0	0.50	0.49	0.53
		1	0.41	0.42	0.44
		2	0.32	0.33	0.38
		3	0.25	0.25	0.28
Light petroleum (b.p. 120–160°)	0.75	0	0.48	0.50	0.49
		1	0.43	0.43	0.43
		2	0.43	0.43	0.43
		3	0.41	0.42	0.41
Cyclohexane (b.p. 81°)	0.78	0	0.37	0.37	0.39
		1	0.29	0.30	0.32
		2	0.24	0.26	0.27
		3	0.17	0.20	0.21

GLC elution patterns of a series of saturated and unsaturated halides from soya bean oil methyl esters are shown in Fig. 1. These patterns are similar to those of the corresponding methyl esters, methyl ketones and acetates. The retention times

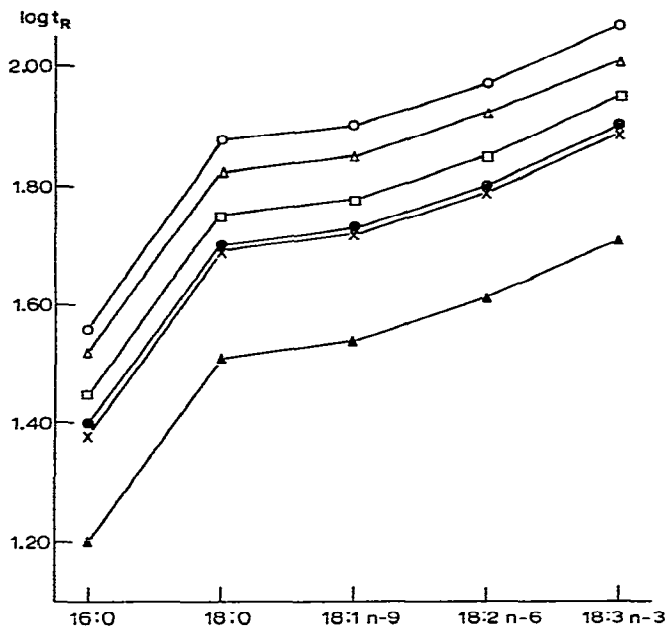


Fig. 1. Schematic representation of GLC elution sequences of long-chain derivatives. ○, Iodides; △, acetates; □, methyl ketones; ●, methyl esters; ×, bromides; ▲, chlorides.

of the halides increased in the order chlorides < bromides < iodides which is the order of decreasing volatility. The methyl esters, methyl ketones and acetates have retention times between those of the corresponding bromides and iodides.

Relative retention data and equivalent chain length (ECL) values for halides, methyl esters, methyl ketones and acetates are given in Table II. A change in the end-group function does not markedly affect the ELC values; the iodide ECL values being slightly lower than those of the corresponding chlorides and bromides. Type II separation factors are given in Table III and these factors are independent of the end-group function but, as expected, change with the polarity of the stationary phase.

## DISCUSSION

### TLC

The separation of long-chain alkyl halides into unsaturation classes by silica gel TLC using hydrocarbon mobile phases is unusual and might not have been predicted since analogous separations of methyl esters or hydrocarbons are best achieved using silver nitrate impregnated adsorbents. It was expected that the interactions of alkyl halides with a silica surface would not be so strong as those of methyl esters but stronger than those of hydrocarbons. This is borne out by the fact that, on silica gel TLC with pentane as the mobile phase, 1-iodooctadecane has an  $R_F$  of 0.75, whilst methyl octadecanoate and octadecane have  $R_F$  values of 0.10 and 0.95, respectively.

With hydrocarbon solutes and hydrocarbon mobile phases the interaction of

TABLE II  
GLC RETENTION DATA FOR LONG-CHAIN COMPOUNDS

Compounds	Column*			
	1		2	
	$t_R$	ECL	$t_R$	ECL
<i>Chlorides</i>				
16:0	0.519	16.00	0.492	16.00
18:0	1.00	18.00	1.00	18.00
18:1 <i>n</i> - 9	1.11	18.32	1.08	18.22
18:2 <i>n</i> - 6	1.38	18.98	1.27	18.67
18:3 <i>n</i> - 3	1.82	19.83	1.62	19.33
<i>Bromides</i>				
16:0	0.519	16.00	0.495	16.00
18:0	1.00	18.00	1.00	18.00
18:1 <i>n</i> - 9	1.12	18.35	1.07	18.19
18:2 <i>n</i> - 6	1.40	19.03	1.27	18.68
18:3 <i>n</i> - 3	1.83	19.84	1.60	19.34
<i>Iodides</i>				
16:0	0.516	16.00	0.497	16.00
18:0	1.00	18.00	1.00	18.00
18:1 <i>n</i> - 9	1.12	18.34	1.05	18.14
18:2 <i>n</i> - 6	1.38	18.98	1.25	18.64
18:3 <i>n</i> - 3	1.81	19.80	1.55	19.25
<i>Methyl esters</i>				
16:0	0.522	16.00	0.503	16.00
18:0	1.00	18.00	1.00	18.00
18:1 <i>n</i> - 9	1.11	18.32	1.07	18.20
18:2 <i>n</i> - 6	1.38	18.99	1.25	18.65
18:3 <i>n</i> - 3	1.83	19.86	1.55	19.27
<i>Methyl ketones</i>				
16:0	0.520	16.00	0.500	16.00
18:0	1.00	18.00	1.00	18.00
18:1 <i>n</i> - 9	1.12	18.35	1.06	18.20
18:2 <i>n</i> - 6	1.41	19.05	1.26	18.67
18:3 <i>n</i> - 3	1.89	19.95	1.58	19.32
<i>Acetates</i>				
16:0	0.513	16.00	0.500	16.00
18:0	1.00	18.00	1.00	18.00
18:1 <i>n</i> - 9	1.10	18.29	1.07	18.20
18:2 <i>n</i> - 6	1.38	18.97	1.25	18.66
18:3 <i>n</i> - 3	1.81	19.78	1.55	19.30

\* See Experimental.

the solute with the mobile phase is much stronger than that with the silica surface and, under these conditions, high  $R_F$  values but no separation into unsaturation classes are obtained. With methyl ester solutes there is a low solubility in the hydrocarbon mobile phase and a strong interaction of the ester group with the silanol groups on the silica surface<sup>17,18</sup>. The interactions of an ester group would be expected to be much stronger than those of  $\pi$ -electrons of an olefinic group with the silanol groups, and again no separation into unsaturation classes would be expected. However, with a more polar solvent, it is possible to separate long-chain acetylenic methyl esters from olefinic esters on silica gel.

TABLE III  
TYPE II SEPARATION FACTORS

Compounds	Column*			
	1		2	
	18:3 n - 3/ 18:2 n - 6	18:2 n - 6/ 18:1 n - 9	18:3 n - 3/ 18:2 n - 6	18:2 n - 6/ 18:1 n - 9
Chlorides	1.32	1.24	1.26	1.18
Bromides	1.31	1.25	1.26	1.19
Iodides	1.31	1.23	1.25	1.19
Methyl esters	1.33	1.24	1.24	1.17
Methyl ketones	1.34	1.26	1.25	1.18
Acetates	1.31	1.26	1.24	1.17

\* See Experimental.

Even the olefinic band obtained has the more saturated esters concentrated in the leading edge and those esters with the greatest olefinic unsaturation concentrated in the trailing edge<sup>19</sup>.

Both the halogen atom and the olefinic  $\pi$ -electrons of an unsaturated long-chain halide may interact to a similar extent with the silanol groups of a silica surface and, when an alkane mobile phase is used, separation into unsaturation classes would be possible. The separations which were obtained were carried out on silica gel without binder and when this material was made more active after additional heating, lower  $R_F$  values were obtained but the separation into unsaturation classes was unaltered. This may be explained by the presence of an increased number of silanol sites on the surface of the more active silica gel. Separation into unsaturation classes was not obtained either with silica gel containing calcium sulphate nor with alumina.

The  $R_F$  values of the saturated halides decreased with increasing density of the mobile phase. The separation into unsaturation classes also decreased with increasing density of the mobile phase except that, with cyclohexane, a similar separation was obtained to that with petroleum ether (b.p. 80–100°), *i.e.*, the effectiveness of the separation was more dependent on the volatility of the mobile phase. Scott<sup>20</sup> has discussed the variation of retention times with solvent density and showed that the use of mobile phases of different densities can cause significant changes in elution sequence particularly when the polar interactions of the solute and the two phases are relatively weak, this would be the situation with long-chain olefinic halides, a silica gel surface and a saturated hydrocarbon mobile phase.

With mobile phases which gave good separation into unsaturation classes the  $R_F$  values in each unsaturated class increased in the order chloride < bromide < iodide. It would be expected that, with increasing size of the halogen, the interaction with the silanol groups on the silica surface would decrease.

### GLC

The elution sequence of the long-chain compounds on a polyester column is chloride < bromide < methyl ester < methyl ketone < acetate < iodide, which is the order of decreasing volatility. Since this sequence is the same for saturated and each unsaturation class of compound, and, since the relative retention data are similar

for each functional group series, then, at a particular column temperature, the separation between saturated compounds and each unsaturation class is virtually independent of the functional end-group and depends only on the number and positions of the olefinic bonds. This is also illustrated by the Type II separation factors which are similar for halides, methyl esters, methyl ketones and acetates. These Type II factors indicate the change in retention behaviour when additional unsaturation is inserted near the methyl end of a hydrocarbon chain and would be expected to be independent of the functional end-group, provided that this group is not too bulky<sup>21</sup>.

#### CONCLUSIONS

The novel TLC characteristics of long-chain alkyl halides, found under certain prescribed conditions, may be explained by considering the interactions of olefinic  $\pi$ -electrons and halide atoms with the silanol groups of a silica gel surface. The GLC characteristics of these long-chain halides are similar to those of the corresponding methyl esters, methyl ketones and acetates. The combined TLC and GLC data should help in the isolation and identification of small amounts of alkyl halides obtained from the alkyl chains of glyceryl-1-ethers. In addition to basic structural studies in ether lipids, the data could be useful in the rapidly expanding area of pheromone synthesis where long-chain halides are useful intermediates.

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