

## Note

### Gas-liquid chromatography characteristics of some long-chain methyl ketones

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Long-chain methyl ketones have been found as constituents of the lipids from a wide variety of natural sources, e.g. peat and soil waxes<sup>1,2</sup>, snake skin<sup>3</sup>, marine crustacea<sup>4</sup>, insect pheromones<sup>5,6</sup>. Recently, Jamieson and Reid<sup>7</sup> established the presence of long-chain acetylenic methyl ketones and the corresponding acids in the lipids of the moss, *Fontinalis antipyretica* and a preliminary investigation indicated that these acetylenic compounds were present in a related moss species, *F. dalecardia*<sup>8</sup>.

Current studies in the developing area of pheromone chemistry indicate that gas chromatography can be of great value for the elucidation of the structures of long-chain compounds provided that adequate background retention data are available. The use of gas chromatography as an aid in the identification of long-chain methyl esters is now well established<sup>9-11</sup> and it has also been shown<sup>12,13</sup> that the method can be used when the  $-\text{CO}_2\text{Me}$  group is replaced by  $-\text{CH}_2\text{OH}$ ,  $-\text{CH}_2\text{OAc}$ , and  $-\text{CH}_3$  and also when a non-methylene-interrupted olefinic centre is present in polyolefinic methyl esters.

In the present work, retention characteristics of long-chain methyl ketones have been determined and used to extend, to these methyl ketones, the system of identification established for methyl esters.

#### EXPERIMENTAL AND RESULTS

Methyl ketones were obtained by the reaction of methyl lithium<sup>14</sup> with the long-chain fatty acids obtained from a variety of seed and marine lipids. Gas-liquid chromatography was carried out with nitrogen as the mobile phase on the following 6 ft.  $\times$  1/8 in. I.D. stainless-steel columns: (1) 5% BDS, 160°; (2) 5% DEGS, 180°; (3) 10% EGSS-X, 190°; (4) 10% EGSS-X, 210°.

#### Retention data

Relative retention data and equivalent chain length (ECL) values for a number of olefinic and acetylenic methyl ketones and methyl esters chromatographed on four columns of different polarity are given in Table I. On each of these columns the elution patterns of methyl ketones were similar to those of the corresponding methyl esters but the ketones had slightly greater retention times. ECL values for

TABLE I

## RETENTION DATA OF METHYL KETONES (MK) AND METHYL ESTERS (ME)

	Column 1*				Column 2				Column 3				Column 4			
	MK		ME		MK		ME		MK		ME		MK		ME	
	$t_R$ **	ECL	$t_R$ **	ECL	$t_R$	ECL	$t_R$	ECL	$t_R$	ECL	$t_R$	ECL	$t_R$	ECL	$t_R$	ECL
18:1 n-9	1.08	18.20	1.06	18.15	1.14	18.49	1.10	18.36	1.19	18.60	1.17	18.54	1.20	18.58	1.16	18.33
18:2 n-6	1.25	18.57	1.25	18.57	1.39	19.22	1.38	19.20	1.53	19.48	1.51	19.40	1.55	19.70	1.50	19.53
18:3 n-6	1.41	18.88	1.40	18.86	1.65	19.87	1.61	19.77	1.84	20.12	1.82	20.04	1.86	20.41	1.79	20.20
18:3 n-3	1.59	19.19	1.59	19.18	1.81	20.21	1.78	20.14	2.07	20.53	2.04	20.43	2.05	20.78	2.00	20.62
18:4 n-3	1.80	19.51	1.80	19.50	2.15	20.85	2.08	20.71	2.54	21.24	2.42	21.01	2.47	21.51	2.38	21.28
20:2 n-6	2.67	20.57	2.64	20.50	2.37	21.21	2.28	21.06	2.67	21.41	2.62	21.28	2.54	21.59	2.52	21.52
20:3 n-6	2.93	20.76	2.88	20.69	2.66	21.65	2.58	21.54	3.15	21.99	3.09	21.84	2.95	22.16	2.92	22.09
20:3 n-3	3.44	21.18	3.31	21.05	3.07	22.18	2.97	22.04	3.64	22.49	3.56	22.33	3.32	22.65	3.31	22.52
20:4 n-6	3.13	20.93	3.10	20.88	3.07	22.18	2.97	22.04	3.76	22.60	3.73	22.48	3.52	22.88	3.43	22.66
20:4 n-3	3.70	21.36	3.60	21.26	3.54	22.71	3.43	22.58	4.35	23.01	4.35	23.01	4.03	23.41	4.01	23.24
20:5 n-3	3.92	21.51	3.88	21.45	3.95	23.12	3.92	23.07	4.88	23.57	4.82	23.36	4.68	23.98	4.57	23.74
22:4 n-6	6.64	22.82	6.41	22.79	5.10	24.07	5.08	24.03	6.70	24.61	6.57	24.41	5.85	24.97	5.79	24.63
22:5 n-6	7.09	22.99	6.98	22.96	5.69	24.48	5.65	24.43	7.52	24.94	7.34	24.87	6.64	25.34	6.43	25.15
22:5 n-3	8.46	23.44	8.27	23.43	6.66	25.07	6.56	24.98	8.94	25.61	8.65	25.35	7.61	25.88	7.52	25.66
22:6 n-3	8.98	23.61	8.87	23.59	7.27	25.40	7.28	25.34	10.08	25.93	9.80	25.87	8.84	26.51	8.48	26.22
18:2 6a, 9	2.22	20.05	2.18	19.99	2.29	21.09	2.23	20.94	2.81	21.59	2.63	21.29	2.71	21.79	2.60	21.71
18:3 6a, 9, 12	2.59	20.45	2.54	20.37	2.78	21.91	2.78	21.80	3.58	22.43	3.48	22.24	3.46	22.81	3.35	21.57
18:4 6a, 9, 12, 15	3.29	21.06	3.23	21.00	3.78	22.96	3.63	22.79	4.88	23.51	4.76	23.31	4.67	23.96	4.46	23.63
20:3 8a, 11, 14	5.25	22.26	5.14	22.17	4.72	23.79	4.64	23.70	6.25	24.37	6.70	24.22	5.55	24.65	5.38	24.36
20:4 8a, 11, 14, 17	7.19	23.03	6.76	22.91	6.05	24.71	5.97	24.63	8.28	25.28	8.14	25.20	7.54	25.80	7.34	25.67
Retention time (min)																
18:0		26.5		22.7		9.5		8.3		7.5		6.5		4.3		3.9

\* Specified in text.

\*\* Relative to 2-ketnonadecane = 1.00.

\*\*\* Relative to methyl octadecanoate = 1.00.

methyl ketones were calculated using saturated straight-chain methyl ketones as reference compounds and ECL values for methyl esters were calculated as described previously<sup>10,11</sup>. The ECL values for methyl ketones ( $ECL_{MK}$ ) calculated in this way were slightly greater than those for the methyl esters ( $ECL_{ME}$ ). The differences,  $ECL_{MK} - ECL_{ME}$ , did increase slightly with increasing column polarity, the mean difference increasing from 0.04 ECL units on the lowest polarity column to 0.20 on the highest polarity column.

It is possible to use the change in ECL values of a particular polyolefinic compound on going from a high to a low polarity column to estimate the degree of unsaturation of that particular compound<sup>13</sup>. From the changes in ECL values obtained from columns 1 and 4 it can be calculated that, in polyolefinic methyl ketones, the contribution of an olefinic group is 0.50 ECL units, and for methyl esters, 0.46 units. The corresponding values for an acetylenic group are 1.31 for methyl ketones and 1.22 for methyl esters. These contributions of an acetylenic group to the variation of retention with polarity of the stationary phase are 2.62 and 2.65 times that of an olefinic group. A similar result was obtained for methyl esters by Hofstetter *et al.*<sup>15</sup> from ECL values on a polar and a non-polar stationary phase and by Jamieson and Reid<sup>7</sup> from ECL values on high and low polarity stationary phases.

#### *Separation factors*

Systematic relationships between retention times of methylene-interrupted polyolefinic methyl esters were first published by Ackman<sup>16</sup> who correlated the structural variables of these esters, *i.e.* chain length, number and position of olefinic groups, to give three types of separation factors. Separation factors have since been used extensively in the identification of unknown methyl esters from plant and marine lipids and their use has been extended to other long-chain compounds.

Since there is a close similarity among the relative retention times of methyl ketones and methyl esters it was expected that there would be an agreement among the respective separation factors. These separation factors are given in Table II. It was found that, for all the types of separation factors, similar values were obtained for methyl ketones and methyl esters. The effects on Types I and II separation factors of introducing an acetylenic group into the "carboxyl end-chain" of a polyolefinic system have been discussed previously<sup>17</sup> and an additional separation factor, Type IIIA is given. This factor shows the effect of replacing an olefinic group at the "carboxyl end" of the chain with an acetylenic group and indicates the increased interaction of the acetylenic group with the stationary phase.

#### *ECL values and stationary phase polarity*

Details of the nature and quantity of the stationary phase, the support material and the operating conditions have traditionally provided an indication, at least to the experienced chromatographer, of the apparent polarity of a gas chromatographic column. Column polarity has never been adequately defined although this has not been through lack of application<sup>18</sup>. It has been suggested that for fatty acid methyl esters and related compounds, column polarity is defined as the degree of separation on a particular column of an unsaturated molecule relative to its saturated analogue. The extent of this separation for a particular column will be dependent



TABLE II (continued)

		Column							
		1		2		3		4	
		MK	ME	MK	ME	MK	ME	MK	ME
<i>Type III</i>									
22:5	7, 10, 13, 16, 19	1.19	1.18	1.17	1.16	1.19	1.18	1.15	1.17
22:5	4, 7, 10, 13, 16								
20:4	8, 11, 14, 17	1.18	1.16	1.20	1.20	1.16	1.17	1.15	1.17
20:4	5, 8, 11, 14								
18:3	9, 12, 15	1.13	1.13	1.15	1.13	1.13	1.14	1.10	1.12
18:3	6, 9, 12								
<i>Type III A</i>									
18:4	6a, 9, 12, 15	1.83	1.80	1.88	1.91	1.92	1.96	1.90	1.97
18:4	6, 9, 12, 15								
18:3	6a, 9, 12	1.84	1.81	1.91	1.91	1.98	1.98	1.90	1.92
18:3	6, 9, 12								
20:3	8a, 11, 14	1.79	1.79	1.91	1.91	1.93	1.87	1.89	1.82
20:3	8, 11, 14								
20:4	8a, 11, 14, 17	1.83	1.87	1.92	1.92	1.95	1.88	1.90	1.83
20:4	8, 11, 14, 17								

principally on the structure of the stationary phase and also on a series of variable parameters such as the loading of the stationary phase, operating temperature etc. In the case of fatty acid methyl esters and related compounds column polarity has been characterised by the ECL value of an arbitrarily chosen unsaturated standard (18:3  $n-3$  is most useful). A high polarity column will have a high  $ECL_{18:3\ n-3}$  and a low polarity column will have a low value.

In recent years one of the most useful approaches towards a general treatment of polyester liquid phase polarity has been to consider polyesters, not as a broad range of different types, but rather as a single class of substance whose polarity will depend on a variety of chromatographic factors. A general ECL relationship has been developed in the form:

$$ECL_X = a_X ECL_{18:3\ n-3} + b_X$$

where  $a_X$  and  $b_X$  are computer-derived constants for the fatty acid methyl ester  $X$ <sup>19</sup>. This relationship was obtained by processing retention data for a series of *cis,cis*-methylene-interrupted polyolefinic esters. Tables of ECL values for each of the esters corresponding to a range of  $ECL_{18:3\ n-3}$  were compiled and sections of these tables and their application to fatty acid analysis have recently been discussed<sup>11</sup>.

One of the most useful applications of this approach has been the correlation of the slope constant  $a_X$  with the degree of unsaturation of unusual polyolefinic-acetylenic methyl esters<sup>18</sup>. In the present work a similar procedure is applied to long-chain methyl ketones and a comparison is made of the computer-derived constants for a series of polyunsaturated methyl ketones and the corresponding constants for methyl esters.

TABLE III  
COMPUTER DERIVED CONSTANTS FOR POLYOLEFINIC COMPOUNDS

<i>Polyolefin</i>	<i>MK</i>			<i>ME</i>		
	<i>a</i>	<i>b</i>	<i>No. of results</i>	<i>a</i>	<i>b</i>	<i>No. of results</i>
18:3 <i>n</i> -6	0.928	1.091	31	0.909	1.480	68
18:4 <i>n</i> -3	1.257	-4.598	22	1.258	-4.661	77
20:2 <i>n</i> -6	0.714	6.767	15	0.647	8.127	62
20:3 <i>n</i> -6	0.882	3.820	15	0.915	3.208	65
20:3 <i>n</i> -3	0.945	3.013	10	0.986	2.189	17
20:4 <i>n</i> -6	1.221	-2.508	14	1.119	-0.509	67
20:4 <i>n</i> -3	1.346	-4.527	17	1.350	-4.606	62
20:5 <i>n</i> -3	1.554	-8.320	17	1.518	-7.602	73
22:4 <i>n</i> -6	1.352	-3.124	10	1.266	-1.448	33
22:5 <i>n</i> -6	1.451	-4.833	6	1.441	-4.600	22
22:5 <i>n</i> -3	1.627	-7.851	11	1.527	-5.843	38
22:6 <i>n</i> -3	1.732	-9.585	9	1.792	-10.726	40

The variations with column polarity of the ECL values of a series of polyolefinic methyl ketones were computer processed and the computer-derived constants for these ketones are given, along with previously published data for methyl esters in Table III. The computer-derived constants for a series of acetylenic methyl ketones and methyl esters are given in Table IV. The constants for the methyl esters were derived from a larger number of samples than had been used previously<sup>17</sup>. There is good agreement of the slope values (*a*) for the methyl ketones and those of the corresponding methyl esters. The effect of changing the functional group at the "carboxyl end" of the molecule does not markedly alter these slope values.

TABLE IV  
COMPUTER DERIVED CONSTANTS FOR ACETYLENIC COMPOUNDS

<i>Acetylenic</i>	<i>MK</i>			<i>ME</i>		
	<i>a</i>	<i>b</i>	<i>No. of results</i>	<i>a</i>	<i>b</i>	<i>No. of results</i>
18:2 6a, 9	1.092	-0.978	19	1.198	-3.029	25
18:3 6a, 9, 12	1.522	-8.811	40	1.531 (1.51)*	-8.992	61
18:4 6a, 9, 12, 15	1.819	-13.871	26	1.836 (1.75)*	-14.210	26
20:3 8a, 11, 14	1.484	-6.192	30	1.509 (1.50)*	-6.738	54
20:4 8a, 11, 14, 17	1.976	-15.247	6	1.851	-12.745	13

\* Ref. 17.

Since the slope values are dependent on the degree of unsaturation the increments due to an olefinic group were calculated and these are shown in Table V. The mean incremental values were similar for both methyl ketones and methyl esters and these mean values were used to calculate the increments due to an ace-

TABLE V  
INCREMENTS OF SLOPE VALUES ( $a$ ) FOR POLYOLEFINIC METHYL KETONES AND METHYL ESTERS

No. of olefinic groups	Mean slope value ( $a$ )		Increment $a$ per olefinic group	
	MK	ME	MK	ME
2	0.714	0.647	0.357	0.323
3	0.918	0.936	0.306	0.312
4	1.275	1.248	0.309	0.316
5	1.544	1.495	0.309	0.299
6	1.732	1.792	0.289	0.298
		Mean	0.316	0.310

tylenic group (Table VI). The increment due to an acetylenic group was 2.65 times that for an olefinic group in methyl ketones and 2.92 in methyl esters. These values are larger than those obtained from differences in ECL values using only two columns of different polarities but similar to those obtained for acetylenic methyl esters by Hoffstetter *et al.*<sup>15</sup> and by Jamieson and Reid<sup>7</sup>.

TABLE VI  
INCREMENTS OF SLOPE VALUES ( $a$ ) FOR ACETYLENIC METHYL KETONES AND METHYL ESTERS

	MK			ME		
	$a$ (total)	$a$ (olefinic)	$a$ (acetylenic)	$a$ (total)	$a$ (olefinic)	$a$ (acetylenic)
18:2 6a, 9	1.092	0.316	0.776	1.198	0.310	0.888
18:3 6a, 9, 12	1.522	0.632	0.890	1.531	0.620	0.911
20:3 6a, 11, 14	1.464	0.632	0.852	1.509	0.620	0.889
18:4 6a, 9, 12, 15	1.819	0.948	0.871	1.836	0.930	0.906
20:4 8a, 11, 14, 17	1.742	0.948	0.794	1.851	0.930	0.921
		Mean	0.837			0.905

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#### REFERENCES

- 1 R. I. Morrison and W. Bick, *Chem. Ind. (London)*, (1966) 596.
- 2 R. I. Morrison and W. Bick, *J. Sci. Food Agr.*, 18 (1967) 351.
- 3 D. G. Ahern and D. T. Downing, *Lipids*, 9 (1974) 8.
- 4 K. Kanada, H. Seino, S. Watanabe and Y. Abe, *Yukagaku*, 22 (1973) 654.
- 5 W. L. Roelofs and R. T. Caroe, in M. C. Birch (Editor), *Pheromones*, American Elsevier, New York, 1974, p. 96.

- 6 J. M. Luby, F. E. Regnier, E. T. Clarke, E. C. Weaver and E. B. Weaver, *J. Insect Physiol.*, 19 (1973) 1111.
- 7 G. R. Jamieson and E. H. Reid, *Phytochemistry*, 15 (1976) 1731.
- 8 G. R. Jamieson and A. L. McMinn, unpublished results.
- 9 R. G. Ackman, *Progr. Chem. Fats Other Lipids*, 12 (1972) 165.
- 10 G. R. Jamieson, in F. D. Gunstone (Editor), *Topics in Lipid Chemistry*, Vol. 1, Logos Press, London, 1970, p. 167.
- 11 G. R. Jamieson, *J. Chromatogr. Sci.*, 13 (1975) 491.
- 12 G. R. Jamieson and E. H. Reid, *J. Chromatogr.*, 26 (1967) 8.
- 13 G. R. Jamieson and E. H. Reid, *J. Chromatogr.*, 61 (1971) 346.
- 14 M. J. Jorgenson, *Org. React.*, 18 (1970) 1.
- 15 H. H. Hofstetter, H. Sen and R. T. Holman, *J. Amer. Oil. Chem. Soc.*, 42 (1965) 537.
- 16 R. G. Ackman, *Nature (London)*, 194 (1962) 970.
- 17 G. R. Jamieson and E. H. Reid, *J. Chromatogr.*, 128 (1976) 193.
- 18 P. Alessi, I. Kikic and A. Papo, *J. Chromatogr.*, 131 (1977) 31.
- 19 G. R. Jamieson and E. H. Reid, *J. Chromatogr.*, 42 (1969) 304.