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Gas-Liquid Chromatography of Straight-Chain Saturated Wax Esters (95)

(Chokusa Howa Wax No Gas Chromatography)

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The straight-chain saturated wax esters of carbon number from 16 to 44 were synthesized, and separated by gas-liquid chromatography. A 75 cm stainless steel column containing 5% SE-30 on 60/80 mesh Celite 545 resolved wax esters differing by only one carbon number. Relative retention time and responses for the wax esters were calculated.

The fully hydrogenated sperm whale wax esters were analyzed by this technique. The wax esters of carbon number 26 to 42 were present, and the major fractions were C_{32}, C_{34}, C_{36} and C_{38}. Moreover, the composition of the wax esters was calculated from fatty acid and alcohol compositions, assuming ran dom combination. The values nearly agreed with the observed gas-liquid chromatographic results of the chain length composition of the wax esters.

1. Preface

Composition analysis of waxes existing in nature has been performed by means of Gas Chromatography (GC) i.e. the isolated wax is hydrolyzed, and the methyl esters or acetates derived from the resulting fatty acids or alcohols are subjected to GC analysis. The data obtained are used in discussion of wax structure. At present, many reports[1)] have been published about the practical applications of GC analysis to several materials with high boiling points such as triglycerides and sterines etc., owing to the

development of low liquid-phase columns and high-sensitivity detectors. Very few experimental reports[2,3] were found on the direct GC analysis of wax, although it should have been possible technically. In proceeding with the previous study on the separation and analysis of wax by means of thin-layer chromatography[4], the authors will present the results of direct GC analysis of synthetic straight-chain saturated wax esters with carbon numbers ranging from 16 to 44 and sperm whale wax esters.

2. Experiments

The synthetic saturated wax esters used in the experiments are listed in Table I. The method of synthesizing wax esters was described previously[4]. (96) The wax esters were all refined by means of thin-layer chromatography. They contained no other components but wac esters, but the wax homologue was mixed, and the purity of the main component was 96.5-99.3%. The sperm whale wax esters were obtained by the following procedure. Commercial sperm whale oil was fractioned by silica gel column chromatography. The wax fraction obtained was further purified by thin layer chromatography to yield a pure ester. Moreover, before use in experiments they were fully hydrogenated in a hexane solution with a palladium carbon catalyst until they showed a zero iodine value. Table 2 shows the chain-length composition of alcohols and fatty acids from of sperm whale wax esters by means of GC analysis. The GC analysis of wax esters was performed under the following conditions.

Apparatus:	YANAGIMOTO GCG 550F Model
Detector:	Hydrogen flame ionization detector
Column:	75 cm x 3 mm I.D., U shaped stainless steel column
Packing:	SE-30 (5% on 60-80 mesh Celite 545), (silanized)
Column Temperature:	Programed from 170°C to 320°C at a rate of 4°C/min. Moreover, isothermal analysis was performed arbitary in the range of 180-300°C.
Carrier Gas:	Nitrogen, inlet pressure 0.5 kg/cm^2 at 170°C, and 1.0 kg/cm^2 at 320°C.
Combustion Gas:	H_2, 25 ml/min.
Air:	800 ml/min.
Recording Speed:	5 mm/min.
Injected Sample Size:	0.2-5 µl in approximately 2% chloroform solution.
Sensitivity:	10^8.

Peak areas of chromatogram was all evaluated by the half-width method.

Table. 1 Synthetic saturated wax esters and their relative retention time.

Carbon number	Ester	Relative retention time* (Column temp. °C) 180	230	280
16	Octylcaprylate	0.050		
17	Octylpelargonate	0.075		
18	Octylcaprate	0.110		
19	Decylpelargonate	0.155		
20	Decylcaprate	0.225		
21	Decylhendecylate	0.325		
22	Decyllaurate	0.475		
23	Hendecyllaurate	0.700		
24	Lauryllaurate	(1.00)	(1.00)	
25	Lauryltridecylate		1.35	
26	Myristyllaurate		1.70	
27	Myristyltridecylate		2.20	
28	Myristylmyristate		2.90	
29	Myristylpentadecylate		3.85	
30	Cetylmyristate		5.00	
31	Cetylpentadecylate		6.00	
32	Cetylpalmitate		8.60	(1.00)
33	Cetylmargarate			1.22
34	Stearylpalmitate			1.50
35	Stearylmargarate			1.83
36	Stearylstearate			2.28
37	Stearylnonadecylate			2.78
38	Stearylarachidate			3.39
39	Arachylnonadecylate			4.17
40	Arachylarachidate			5.11
42	Docosylarachidate			7.67
44	Docosylbehenate			11.5

* GLC conditions are the same as shown in Fig. 1.

Table. 2 Chain-length composition of alcohols and acids from fully hydrogenated sperm whale wax esters.

Carbon number	Alcohol mole %	Acid mole %
10		0.4
12		3.5
14	3.6	11.8
15	0.7	0.4
16	36.1	26.5
17	0.8	0.6
18	54.4	31.0
20	4.4	19.0
22		6.5

GLD conditions: 1.5m×3mm stainless steel column packed with 10% polydiethylene glycol succinate on Daiasolid S(80/100 mesh); ca. 50 ml/min nitrogen carrier gas; column temperature 205° C; hydrogen flame ionization detector.

3. Experiment Results and Discussion

3. 1 Qualitative and Quantitative Analysis of Synthetic Wax Esters

First of all, separation was attempted at constant column temperatures. (97)

As a result, we learned that the C_{16}-C_{24}, short-chain wax esters separated

at 170-190°C column temperatures. Likewise, the C_{24}-C_{32} wax esters, and the C_{32}-C_{44}, long-chain wax esters separated in the temperature ranges of 200-240°C, and 250-290°C respectively. For example, Fig. 1 shows the chromatograms obtained at 180°C, 230°C, and 280°C respectively. All of these chromatograms show very symmetric peaks. When the log retention times of wax esters at each temperature obtained from Fig. 1 is ploted vs. the number of carbon atoms, they show relationships as in Fig. 2. If the retention times of C_{24}, and C_{32} wax esters are taken as an indices of 1.00, the relative retention times are shown in Table 1. Moreover, the GC analysis, of a mixture of both synthetic octylbebenate (C_{22}-C_8) and decylarachidate (C_{20}-C_{10}) together with cetylmyristate (C_{14}-C_{16}) showed peak. Based on this fact, if the total number of carbon atoms for a wax ester is the same, so would be the retention time.

Secondly, the full separation of C_{16}- C_{44} wax esters was sought by means of programmed GC. There were some limitations in the range of sample wax esters which could be separated during isothermal GC analysis. However, complete analysis was possible up to C_{44} wax esters if the column temperature was programed. Fig. 3 shows the chromatogram obtained with an initial programed temperature of 170°C, at a rate of 4°C/min. There was no tearing and the peaks were quite uniform in shape. Other program rates of 2°C/min. 9°C/min. were tried. Fig. 4 shows the observed relationship between retention (98) time and the number of carbon atoms in the wax esters. Qualitative analysis is possible using the same conditions of initial programed temperature, program rate, and flow rate of carrier gas etc. Moreover, parallel application

of both programed GC analysis and the previously-mentioned isothermal GC analysis would make precise, separation identification of C_{16}-C_{44} straight-chain saturated wax esters.

Thirdly, in order to perform quantitative analysis, mixed wax esters of known ratios were prepared. Each wax ester with an even number of carbon atoms C_{24}-C_{44} was checked for purity by GC analysis. The chromatograms showed one or two small peaks(homologous waxes) around the main peak. Hence, the purity of the main peak can be determined since the proportional area of the peak gives the proportional weight. Thus, a wax ester mixture with a known weight ratio was prepared, after computing the amounts of homologous waxes mixed separately. This sample was used for analysis, under the same conditions as in Fig. 3. The results shown in Table 3 the average values after seven analyses. The purity of each wax ester analysed is shown by standard deviation. The range of standard deviation is 2-5%, which is acceptable for practical purposes. The relative response of each wax was obtained from seven test repetitions, and the value for C_{32} wax ester was taken as 100. The relationship of the number of carbon atoms in the wax esters vs. relative responses appears roughly linear on the graph.

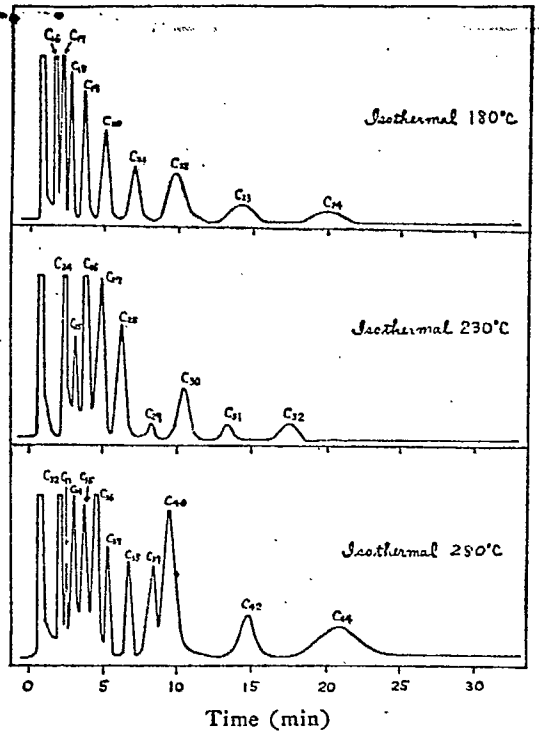

Fig. 1 Gas-liquid chromatograms of straight-chain saturated wax esters.

GLC conditions

Column : 75cm×3mm ID, Stainless steel colum
Packing : 5% SE-30 on 60/80 mesh Celite 545 (silanized)
Column temperature : 180°C, 230°C and 280°C
Carrier gas : nitrogen, 0.78 kg/cm² at 180°C
Chart speed : 5mm/min
Apparatus : YANAGIMOTO GCG 550 F
Detector : Hydrogen flame ionization detector.

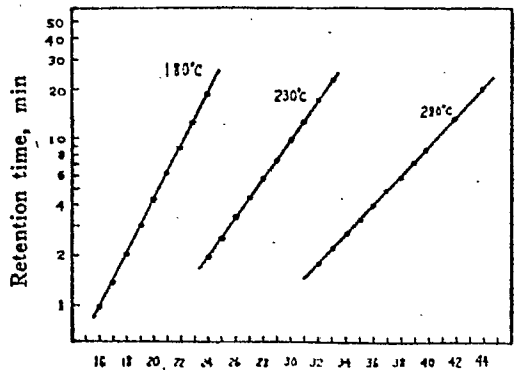

Fig. 2 Relationship between log retention time and number of carbon atoms for the wax esters. The retention times were taken from Fig. 1.

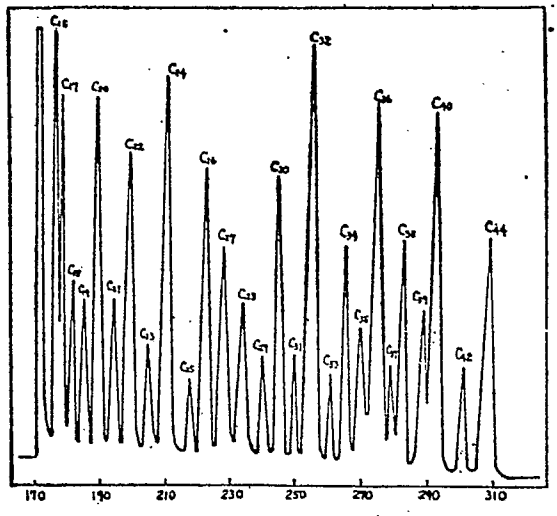

Fig. 3 Gas-liquid chromatogram of straight-chain saturated wax esters.

Column temperature : programed from 170 to 320°C at a rate of 4°C/min, nitrogen carrier gas 0.5kg/cm² at 170°C.

The other conditions are the same as in Fig. 1

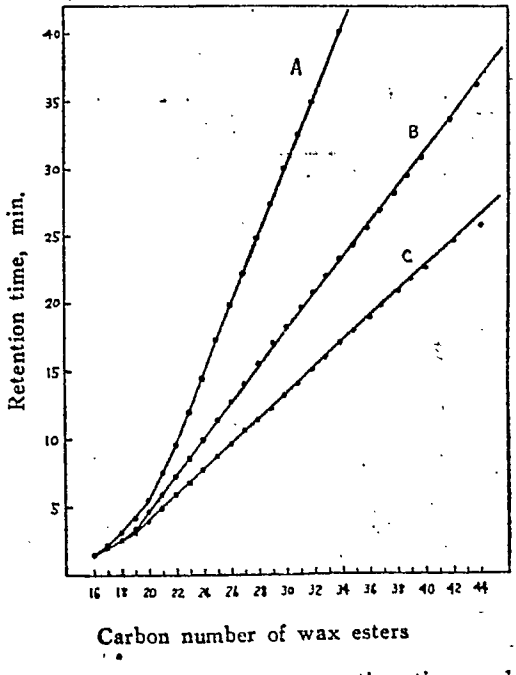

Fig. 4 Relationship between retention time and number of carbon atoms for the wax esters.

Program rate : A. 9 °C/min, B. 4°C/min and C. 2°C/min. The other conditions are the same as in Fig. 3.

Table. 3 Analytical data of the composition of known mixture.

	Carbon number of wax esters										
	24	26	28	30	32	34	36	38	40	42	44
Known-wt %	9.6	9.1	8.6	9.1	9.0	9.6	9.5	9.3	8.9	8.5	8.8
Known-mole %	12.8	11.3	9.9	9.9	9.2	9.3	8.7	8.1	7.4	6.7	6.7
Found-area % (a) *	11.0	10.0	9.2	9.6	9.4	9.5	9.2	8.6	8.7	7.7	7.3
Standard deviation (σ)	0.30	0.25	0.31	0.19	0.26	0.25	0.45	0.27	0.41	0.27	0.43
$\sigma/a \times 100$	2.73	2.51	3.36	2.01	2.79	2.64	4.89	3.14	4.74	3.54	4.97

* Data from seven replicate analysis.

Linear equations can be derived using the least aquares method as done by Mori et al.[5]

Relative Mass Response $R_w = 143.99 - 1.37\ C_n$ (1)

Relative Molar Response $R_m = 72.25 + 0.867\ C_n$ (2)

Here, C_n represents the carbon number of the wax ester.

Relative responses were calculated from Eq. (1) & (2), and shown on Table 4. Also, the elution temperature of each wax ester is added in the Table.

Table. 4 Relative responses and elution temperatures for the wax esters.

Carbon number	Relative responses *		Elution temperature °C
	Mass	Molar	
20	116.42	89.60	186~192
22	113.68	91.33	196~201
24	110.94	83.07	207~212
26	108.21	94.80	219~224
28	105.47	96.54	230~235
30	102.74	98.27	240~245
32	100.00	100.00	251~257
34	97.26	101.74	260~266
36	94.53	103.47	271~276
38	91.79	105.21	278~286
40	89.06	106.93	288~294
42	86.32	108.67	296~301
44	83.58	110.41	305~312

Operating conditions are the same as in Fig. 3.
* Calculated from the equations (1) and (2) in the text.

3.2 Analysis of Sperm Whale Wax Esters

One way in which these techniques can be applied to the analysis of natural waxes is to subject saturated and fully hydrogenated sperm whale wax esters to isothermal or programed GC analysis. One such chromatogram is shown in Fig. 5. Fourteen wax esters, C_{26}-C_{42} were found. In Table 2, the highest carbon number for a fatty acid is C_{22} and for an alcohol C_{20}, leading us to conclude that no wax will have C_{44} or higher. In Fig. 5, C_{32}, C_{34}, C_{36}, and C_{38} waxes appear to be the major components. Small peaks are also evident in the chromatogram, and may be those of waxes with odd carbon numbers. The area of each of the peaks was adjusted in accordance with the relative responses shown in Table 4 and the composition of the wax esters was found. Using the values in Table 2, the wax ester compositions was calculated on the assumption that fatty acids and alcohols combined in a totally random fashion. These results are shown in Table 5. The results of both GC analysis and computations based on fatty acid and alcohol composition were largely the same. Direct GC analysis yielded an average carbon chain length of 34.32 for the synthetic wax esters, which was quite close to the computed value of 34.67. (99)

The authors have separated fractions of sperm whale wax esters with different degrees of unsaturation using silver nitrate chains. As a result, the wax esters samples contained only minute amounts of saturated wax esters and were composed chiefly of mono- and di-unsaturated wax esters. Therefore, the peaks separated at each carbon number in Fig. 5 will be considered the carbon distribution of unsaturated wax esters. There are detailed reports by Mori[5], Okura[6] and others on the fatty acids and alcohols in sperm whale

wax esters. In short, it can be supposed that cetylhexadecenoate is a main component of C_{32} wax esters, as is cetyloleate of C_{34}, oleyloleate of C_{36}, and oleyleicosenate of C_{38}.

Table. 5 Chain-length composition of the wax esters of sperm whale.

Carbon number	GLC detn.* mol %	Calculated** mole %
24		trace
25		trace
26	1.0	0.3
27	0.1	trace
28	2.8	1.9
29	0.1	0.1
30	6.5	7.1
31	0.3	0.4
32	18.9	17.6
33	0.5	0.9
34	28.5	27.1
35	0.4	0.7
36	23.5	25.2
37		0.2
38	12.8	14.2
39		trace
40	4.2	4.3
42	0.3	0.3

* Fully hydrogenated wax esters were chromatographed.

** From the data of Table 2, assuming random combination of the alcohol and acid moieties.

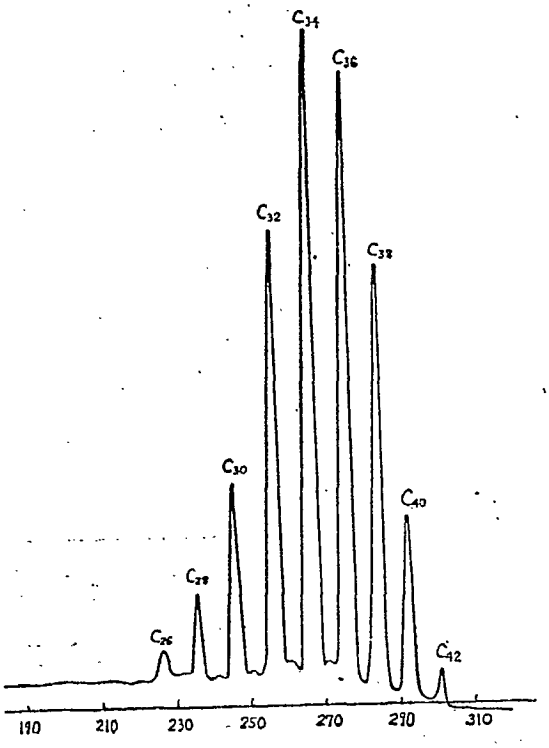

Fig. 5 Gas-liquid chromatogram of fully hydrogenated sperm whale wax esters.
The conditions are the same as in Fig. 3.

3. 3 Limited Application and Several Problems

The authors have attempted[7] GC analysis of a wax ester isolated from the wax insect under the same experimental conditions described in this report, but no distinct peak was obtained higher than C_{48}. Thus, under these conditions analysis is confined to below C_{46}. Moreover, natural waxes are confined to straight chains. No studies were made on mixtures of forked or closed chain wax esters. This problem, as well as separation and quantitative analysis of straight-chain wax esters higher than C_{48} remain to be studied. The maximum carbon number, and whether the straight-chain constitutes the main body or not, can be surmised on the basis of the fatty acid and alcohol components obtained by saponification. This analysis should be made in conjunction with GC analysis of wax esters. Saturated and unsaturated wax esters should be separated at different degrees of unsaturation by the silver nitrate chain method and each fraction GC analyzed until a polar liquid phase suitable for GC analysis is developed.

In closing, the authors would like to express their gratitude to Mr. Kenichi Kishimoto, Section Chief, Yanagimoto Manufacturing Co. for his useful advice in this research.

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(MS received December 20, 1968).