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Alkoxy-Acyl Combinations in the Wax Esters from Winterized Sperm Whale Oil by Gas Chromatography-Mass Spectrometry[1]

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ABSTRACT

Wax esters from winterized sperm whale oil were separated according to degree of unsaturation and then analyzed by gas chromatography-mass spectrometry. The combinations of fatty alcohols and acids making up the wax esters of each chain length were determined. Octadecenyl octadecenoate was the most abundant wax ester (14%), followed by octadecenyl hexadecenoate (10%). Over 240 different wax esters were detected and quantitated.

INTRODUCTION

Sperm whale oil—its composition and outstanding lubricating qualities—has long been intriguing to oil chemists. Efforts to find synthetic replacements for it prompted an in-depth study of its component fatty acids and alcohols (1). Other investigations have been conducted from a biochemical standpoint (2-5). But the fatty acids and alcohols occur combined as wax esters in the oil and important properties have been attributed to these wax esters. So far, a significant question has remained unanswered: what combinations of fatty acids and alcohols make up the wax esters in sperm whale oil? Recent work on jojoba oil (6), a simpler wax ester mixture, showed that the mass spectroscopy (MS) method proposed by Aasen et. al (7) could be used to determine the alcohol-acid combinations in long chain wax esters, and that gas chromatography-mass spectrometry (GS-MS) in a repetitively scanning mode gave reliable results. This paper describes the application of the GC-MS technique to the wax-esters of sperm whale oil.

EXPERIMENTAL PROCEDURES

Sample Preparation and Separation

Two winterized sperm whale oils (Moby Dick 45 NW, Werner G. Smith, Inc. and 40.5 NW Spermoil, Archer Daniels Midland Co.) were investigated. Wax esters were separated from triglycerides on a 1 cm i.d. chromatographic column packed with 10 g of Hi-Flosil (Applied Science Labs., State College, PA) which had been equilibrated with benzene/isooctane (50:50). Approximately 0.5 g of oil was applied to the column and was eluted with 100 ml of the benzene/isooctane mixture followed by 100 ml of hexane/Et_2O (70:30). Preparative layer chromatography (PLC) on 1-mm plates coated with 20% $AgNO_3$ in Silica Gel G separated the wax esters by degrees of unsaturation. The solvent system was benzene/$CHCl_3$ (50:50). Three bands, identified after the plates had been sprayed with a dichlorofluorescein solution, were scraped from the plates, and the components were recovered from the absorbent with Et_2O. Micro-hydrogenations were carried out in toluene/EtOH (1:10) with 10% Pd-on-charcoal as the catalyst.

Preparation of Standard Wax Esters

Acid chlorides were prepared by adding 2 ml of oxalyl chloride (Aldrich Chemical Co., Milwaukee, WI) to 400 mg of the appropriate fatty acid (18:0, 18:1). When the reaction was complete, as indicated by a cessation of bubbling (5 min), the excess oxalyl chloride was removed under N_2. Two acid chlorides (16:0, Eastman, Rochester, NY and 16:1, Nu-Check Prep, Elysian, MN) and four alcohols (16:0, 16:1, 18:0, and 18:1, Nu-Chek-Prep) were purchased. To prepare the wax esters, equimolar amounts of alcohol (in 10 ml of C_6H_6) and acid chloride (in 0.5 ml pyridine) were mixed and allowed to stand 15 min with occasional swirling. The reaction mixture was concentrated on a steam bath under N_2, and wax esters were recovered by PLC on 2-mm layers of Silica Gel with a hexane/Et_2O (70:30) solvent system. High pressure liquid chromatography (HPLC) (6) was used to rid some preparations of small amounts of wax esters with other than the desired chain length, and all were subjected to $AgNO_3$-PLC as described above for final purification.

GC and GC-MS

For GC analyses, a Packard 7401 GC was equipped with a 180 cm x 0.2 mm column packed with 3% OV-1 on Gas Chrom Q (Applied Science Labs.). The column oven was programmed from 240 C to 295 C at 1 C/min. GC-MS equipment, operating conditions, and data aquisition/reduction were essentially the same as previously described (6,8).

RESULTS

Comparison of the analytical data from the two oils and their fractions confirmed the earlier postulation (1) that these oils are virtually identical. Therefore, complete data on only one of them are presented here. The relative proportions of saturated, monoenoid, and dienoid wax esters were estimated from GC analysis of the intact oil and the three fractions, without depending on quantitative recovery from $AgNO_3$-PLC. After $AgNO_3$-PLC, C_{24} wax esters were found only in the saturated fraction, and C_{40} compounds were found only in the dienoid fraction. The area percentage for C_{24} was 18 times greater in the saturated fraction than in the intact oil, whereas the C_{40} percentage was approximately twice greater in the dienoid fraction. These two components could therefore be used as "native" internal standards to estimate the relative abundances of the three classes of unsaturation. Saturated esters represented 6% (100/18) of the oil and dienoid esters 50% (100/2), leaving 44% attributable to monoenoid waxes. Area percentages for individual chain lengths in each fraction were then multiplied by the appropriate factor (0.06 for saturated, 0.44 for monoenoid, and 0.50 for dienoid) to obtain an overall composition for the oil (Table I left column). Agreement was excellent between the sums of the three values for each chain length compared to the composition obtained by GC of the intact oil (1).

Isomer composition according to chain length and degree of unsaturation is also given in Table I. A suggested shorthand notation (7) with the alcohol preceding the acid (e.g., 16:1-18:0) is employed. Isomer composition among the individual classes is discussed in detail below.

[1]Presented at the AOCS Meeting in San Francisco, 1979.

TABLE I

Isomer Composition of the Wax Esters of Commercial Sperm Whale Oil

Chain length and percent[a]	Saturated alc-acid %	Monoenoid[b] alc-acid %	Dienoid alc-acid %
C_{24}	10-14 4		
Saturated = 0.2	11-13 3		
Monoenoid = ---	12-12 6		
Dienoid = ---	13-11 2		
	14-10 72		
	15-9 1		
	16-8 12		
C_{25}	10-15 5		
Saturated = 0.05	11-14 4		
Monoenoid = ---	12-13 3		
Dienoid = ---	13-12 4		
	14-11 18		
	15-10 54		
	16-9 13		
C_{26}	10-16 4	12:0-14:1 6 (5)	
Saturated = 1.5	11-15 3	14:0-12:1 40 (49)	
Monoenoid = 0.3	12-14 3	14:1-12:0 6	
Dienoid = ---	13-13 2	16:0-10:1 26 (46)	
	14-12 33	16:1-10:0 23	
	15-11 4		
	16-10 51		
C_{27}	10-17 5	14:0-13:1 5 (14)	
Saturated = 0.2	11-16 3	14:1-13:0 3	
Monoenoid = 0.04	12-15 3	15:0-12:1 30 (35)	
Dienoid = ---	13-14 3	15:1-12:0 15	
	14-13 7	16:0-11:1 11 (18)	
	15-12 40	16:1-11:0 3	
	16-11 23	17:0-10:1 27 (31)	
	17-10 17	17:1-10:0 4	
C_{28}	12-16 3	12:0-16:1 2 (3)	14-14 17
Saturated = 1.8	13-15 2	14:0-14:1 18 (22)	16-12 70
Monoenoid = 2.1	14-14 13	14:1-14:0 4	18-10 13
Dienoid = 0.3	15-13 4		
	16-12 73	16:0-12:1 30 (48)	
	17-11 1	16:1-12:0 13	
	18-10 5	18:1-10:0 17 (26)	
C_{29}	12-17 3	14:0-15:1 5 (4)	15-14 13
Saturated = 0.3	13-16 3	15:0-14:1 33 (34)	16-13 29
Monoenoid = 0.3	14-15 6	16:0-13:1 18 (26)	17-12 58
Dienoid = 0.05	15-14 17	16:1-13:0 5	
	16-13 24	17:0-12:1 21 (36)	
	17-12 44	17:1-12:0 18	
	18-11 3		
C_{30}	12-18 4	12:0-18:1 2 (3)	14-16 5
Saturated = 0.6	13-17 2	12:1-18:0 3	16-14 47
Monoenoid = 6.7	14-16 11	13-17 --- (1)	18-12 48
Dienoid = 1.4	15-15 2	14:0-16:1 10 (12)	
	16-14 64	14:1-16:0 6	
	17-13 2	15-15 --- tr	
	18-12 17	16:0-14:1 37 (48)	
		16:1-14:0 10	
		17-13 --- (1)	
		18:0-12:1 8 (34)	
		18:1-12:0 25	
C_{31}	12-19 7	14:0-17:1 5 (5)	15-16 5
Saturated = 0.6	13-18 3	15:0-16:1 17 (30)	16-15 8
Monoenoid = 0.6	14-17 5	15:1-16:0 9	17-14 55
Dienoid = 0.2	15-16 12	16:0-15:1 16 (14)	18-13 32
	16-15 25	16:1-15:0 5	
	17-14 32	17:0-14:1 13 (34)	
	18-13 10	17:1-14:0 17	
	19-12 7	18:0-13:1 8 (17)	
		18:1-13:0 8	
C_{32}		13-19 --- (1)	
Saturated = 0.8	12-20 4	14:0-18:1 8 (9)	14-18 3
Monoenoid = 12.0	13-19 4	14:1-18:0 1	15-17 1
Dienoid = 5.2	14-18 6	15-17 --- (1)	16-16 36
	15-17 1	16:0-16:1 44 (50)	18-14 59
	16-16 63	16:1-16:0 9	20-12 1
	17-15 2	17-15 --- (1)	
	18-14 19	18:0-14:1 9 (37)	
	19-13 1	18:1-14:0 27	
	20-12 2	19-13 --- (1)	
		20:1-12:0 1 (1)	

TABLE I (continued)

Isomer Composition of the Wax Esters of Commercial Sperm Whale Oil

Chain length and percent[a]	Saturated alc-acid %	Monoenoid[b] alc-acid %	Dienoid alc-acid %
C_{33}	14-19 19	14:0-19:1 1 (3)	15-18 4
Saturated = 0.06	15-18 2	15:0-18:1 11 / 15:1-18:0 6 (19)	16-17 11
Monoenoid = 0.9	16-17 17		17-16 51
Dienoid = 0.7	17-16 40	16:0-17:1 14 / 16:1-17:0 4 (16)	18-15 22
	18-15 10		19-14 12
	19-14 11	17:1-16:0 18 / 17:0-16:1 15 (33)	
	20-13 1		
		18:0-15:1 7 / 18:1-15:0 14 (21)	
		19:0-14:1 8 (9)	
C_{34}	14-20 10	14:0-20:1 4 (6)	14-20 1
Saturated = 0.1	15-19 4	15-19 --- (1)	16-18 18
Monoenoid = 12.5	16-18 17	16:0-18:1 47 / 16:1-18:0 15 (50)	17-17 3
Dienoid = 13.8	17-17 8		18-16 74
	18-16 46	17-17 --- (4)	20-14 2
	19-15 5	18:0-16:1 9 / 18:1-16:0 23 (34)	
	20-14 11		
		19-15 --- (1)	
		20:1-14:0 2 (3)	
C_{35}	15-20 3	15:0-20:1 8 / 15:1-20:0 7 (16)	15-20 1
Saturated = 0.05	16-19 20		16-19 3
Monoenoid = 1.1	17-18 24	16:0-19:1 5 / 16:1-19:0 6 (11)	17-18 33
Dienoid = 0.8	18-17 11		18-17 35
	19-16 39	17:0-18:1 25 / 17:1-18:0 8 (40)	19-16 25
	20-15 3		20-15 2
		18:0-17:1 10 / 18:1-17:0 10 (16)	
		19:0-16:1 9 / 19:1-16:0 8 (15)	
		20:1-15:0 4 (3)	
C_{36}	16-20 12	14:0-22:1 4 / 14:1-22:0 6 (6)	14-22 2
Saturated = 0.02	17-19 4		16-20 9
Monoenoid = 5.5	18-18 22	16:0-20:1 46 / 16:1-20:0 6 (54)	18-18 87
Dienoid = 15.7	19-17 6		20-16 3
	20-16 44	17-19 --- (1)	
	21-15 4	18:0-18:1 17 / 18:1-18:0 11 (29)	
	22-14 9		
		19-17 --- (2)	
		20:0-16:1 5 / 20:1-16:0 4 (7)	
		21-15 --- (1)	
		22:0-14:1 1 (1)	
C_{37}		15:0-22:1 3 (4)	15-22 2
Saturated = ---		16:0-21:1 3 / 16:1-21:0 5 (4)	17-20 19
Monoenoid = 0.3			18-19 15
Dienoid = 0.5		17:0-20:1 28 / 17:1-20:0 10 (54)	19-18 50
			20-17 7
		18:0-19:1 2 / 18:1-19:0 7 (3)	21-16 7
		19:0-18:1 14 / 19:1-18:0 13 (22)	
		20:0-17:1 8 / 20:1-17:0 1 (6)	
		21:0-16:1 6	
C_{38}		16:0-22:1 36 / 16:1-22:0 7 (45)	16-22 6
Saturated = ---			18-20 75
Monoenoid = 1.6		18:0-20:1 28 / 18:1-20:0 10 (44)	20-18 17
Dienoid = 8.7			22-16 2
		19-19 --- (1)	
		20:0-18:1 6 / 20:1-18:0 4 (7)	
		21-17 --- (1)	
		22:0-16:1 9 (2)	
C_{39}			17-22 6
Saturated = ---			18-21 9
Monoenoid = ---			19-20 61
Dienoid = 0.1			21-18 25
C_{40}			18-22 62
Saturated = ---			20-20 33
Monoenoid = ---			22-18 5
Dienoid = 2.8			

[a]Total number of carbon atoms in the alcohol and acid moieties. Percent figures reflect proportion in intact oil.

[b]Figures in parentheses are taken from fully hydrogenated sample, see text.

TABLE II

Make-up of Known vs. Experimental Values for Monoenoid Wax Ester Standards

Alcohol-acid combination	Standard A			Standard B			Standard C		
	Wt %	Run 1	Run 2	Wt %	Run 1	Run 2	Wt %	Run 1	Run 2
16:0-18:1	9	11	9	41	40	40	27	31	30
16:1-18:0	44	46	45	7	14	12	23	23	24
18:0-16:1	39	34	35	7	8	6	25	19	20
18:1-16:0	8	9	11	45	39	42	25	17	26

Saturated Wax Esters

The saturated wax esters ranged in chain length from C_{24} to C_{36}. Although the major constituents had an even number of carbon atoms (even-chain hereinafter), a good deal of material was eluted between these even chain esters, often appearing as more than one peak (shoulders were common). Areas for these components were combined and considered as representing odd chain esters even though the even chain branched alcohols and acids found in the oil (1) would exist in even chain wax esters if combined with another even chain moiety. Such branched wax esters would probably be eluted before their straight chain counterparts in much the same manner as branched chain methyl esters are eluted before straight chain ones (1). In any event, identities of branched constituents were not established.

Calculations of the proportions of isomers within each chain length were based on the method proposed by Aasen et al. (7). That is, given the general wax ester formula RCO_2R' where R and R′ represent the acid and alcohol groups, respectively, intensities of the ions RCO_2H^+, $RCO_2H_2^+$, and $(R'-1)^+$ were summed for each combination possible within a given chain length. The relative percentage of each sum represents the relative percentage of that combination. As done earlier with jojoba oil (6), all of the scans for each peak were summed to give a total spectrum for the group of wax esters represented by that peak. Here again, contributions due to branched chain constituents were not recognized.

Unsaturated Wax Esters

Determinations of the isomer composition of the monoenoid wax esters presented a new problem, because the procedure worked out by Aasen et al. applies only to saturated compounds (7). Moreover, analysis of the monoenoid wax esters after hydrogenation would not reveal which moiety of the wax ester had been unsaturated, and such information is necessary for a detailed composition. A suggested course (7) involves reduction with deuterium followed by MS, which should give the diagnostic fragment ions 2 AMU higher for the deuterated species. However, corrections for isotopic impurities must be made (7) and, since sperm whale oil contains positional isomers among its fatty acids and alcohols (1), the uncertainty due to isotope effect must be considered (7).

Another approach is to analyze the monoenoid wax esters without derivatization. To check the feasibility of this strategem, four isomeric wax esters (16:0-18:1, 16:1-18:0, 18:0-16:1, and 18:1-16:0) were prepared, purified, and analyzed. Three standards containing these wax esters in known amounts were also made up and analyzed. Intense peaks in unsaturated wax esters occur at $(RCO\text{-}1)^+$ and RCO^+ if the fatty acid is unsaturated and at $(R'-1)^+$ if the alcohol is unsaturated. Ions for the saturated moiety are not nearly so prominet but are nonetheless evident. Intensities of these three ion types were used with the same calculation scheme as for saturated wax esters. The results shown in Table II are for the three standard mixtures.

TABLE III

Percentages of Major Sperm Whale Oil Components Calculated from Table I

Component	Alcohol[a]	Acid[a]
12:0	---	4.3 (2.0)
14:0	3.9 (3.3)	5.1 (6.2)
16:0	21 (28)	5.5 (11)
18:0	4.6 (4.4)	3.1 (1.6)
14:1	---	8.6 (4.8)
16:1	12 (6)	20.1 (17.2)
18:1	45 (43)	27.9 (26.9)
20:1	3.9 (3.8)	10.6 (10.5)
22:1		6.0 (4.6)

[a]Figures in parentheses are calculated from previous data on a saponified mixture (1).

Agreement was generally good between known and experimental values except when 16:1-18:0 was present in a small amount and 16:0-18:1 was predominant (Standard B). This discrepancy is probably due to an ion of moderate intensity (8% of base peak) at *m/e* 222 in the spectrum of 16:0-18:1, which enhances the major diagnostic ion for the 16:1 alcohol moiety (R′-1 = 222) in 16:1-18:0. Other ions that would contribute to errors can no doubt be found because the longer chain alcohol or acyl groups can give rise to fragments of the same *m/e* ratio as critical ions from shorter chain species. This is, in fact, a shortcoming in the MS or GC-MS technique for determining wax ester combinations whether applied to saturated or unsaturated compounds.

Combinations of two odd chain moieties leading to an even chain wax ester could not be estimated among the monoenoid compounds because the major diagnostic ion for an unsaturated, odd chain alcohol at $(R'-1)^+$ is the same as that of the even chain acyl moiety with one less carbon atom $(RCO\text{-}1)^+$. This introduces only a slight error since combinations of two odd chain groups are not prominent. Estimations among the odd chain monoenoid wax esters were attempted because at least one of the species was assumed to be odd.

As a check, a fully hydrogenated sample of the monoenoid wax esters was analyzed, and the results are given in Table I in parentheses. Here the contributions due to combinations of odd chain groups can be seen among the even chain wax esters. Agreements between the total amounts of the isomers calculated before and after hydrogenation were very good.

Dienoid wax esters were far simpler to analyze than the monoenoid because all of the alcohol and acyl groups were assumed to be monoenes. Contributions from the small amounts of dienoid acids and alcohols found in the oil (1) combined with saturated groups were not considered. Therefore, a fully hydrogenated sample yielded the data necessary for isomer composition calculations.

DISCUSSION

Throughout the experiment, an awareness of the complexity of the sperm whale oil mixture and the indirect nature of the analytical procedures made checks on the

TABLE IV

Major Components of Sperm Whale Oil

Alcohol-acid combination		% as calculated from Table I
18:1-18:1		14
18:1-16:1		10
18:1-20:1		6.5
16:0-18:1		5.9
16:0-16:1		5.3
18:1-14:0		3.2
18:1-14:1		3.1
18:1-16:0		2.9
16:0-20:1		2.5
16:0-14:1		2.5
Others	(number)	
Saturated	(94)	6.3
Monoenoid	(89)	22
Dienoid	(52)	17

quality of the results imperative. Some of these have been mentioned above. As a final test, the percentages of some major constituents were calculated using only the data in Table I. In Table III these values are compared with those previously obtained from analysis of the alcohols and acids from a saponified mixture (1). Major areas of disagreement occur at 16:0 and 16:1 alcohols and at 12:0. 16:0, 18:0, and 14:1 acids. Perhaps these discrepancies are due to enhancements of certain diagnostic ions (see section on unsaturated wax esters) in some spectra. Nonetheless, Table III shows all values to be within reason and most of them to be in excellent agreement considering the numerous calculations that have been made.

Table IV lists the 10 most prominent compounds found in this sperm whale oil. Although over 240 different components were detected and quantitated, these 10 make up over 50% of the oil. Still to be defined are the roles played by particular combinations in determining the properties of sperm whale oil and what effect, if any, the odd or branched chain constituents have.

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