# Sperm Whale Oil Analysis by Gas Chromatography and Mass Spectrometry

**G.F. SPENCER** and **W.H. TALLENT**, Northern Regional Research Laboratory,[1]
Peoria, Illinois 61604

## ABSTRACT

Gas liquid chromatography of winterized sperm oil showed that its wax esters with even carbon numbers range from $C_{24}$ to $C_{42}$ and are present in quantities resembling a normal distribution curve with $C_{34}$ as the mean. Between these even-numbered wax esters, ones with odd chain lengths were eluted. Triglycerides, similarly present in a normal distribution pattern, ranged from $C_{42}$ to $C_{58}$ and also included traces of odd chain species. The component acids and alcohols were analyzed by gas chromatography-mass spectrometry, and double bond positions in the monoenoid components were established. Branched chain and odd chain constituents, both saturated and unsaturated, were detected among both alcohols and acids. These moieties, when combined with those having even chains, are responsible for the wax esters and triglycerides with odd carbon numbers.

## INTRODUCTION

Pursuant to the termination by law of U.S. trade in products from the sperm whale, efforts have been underway to develop substitutes for its valuable oil, particularly for lubricant applications (1). In connection with these efforts, knowledge of the precise chemical composition of the specific product having the efficacious properties to be imitated becomes desirable. Data already in the literature are generally confined to oil from either the sperm whale head or blubber (2) or to the wax ester (3), triglyceride (4) or other (5,6) fractions of the oil individually. Winterized sperm oil, the raw material formerly employed in the lubricant industry, was derived from a composite of head and blubber oils by a process that removed the more saturated components of both lipid fractions (7). Accordingly we undertook detailed analysis of this commercial product acquired before it disappeared from the market.

[1]ARS, USDA.

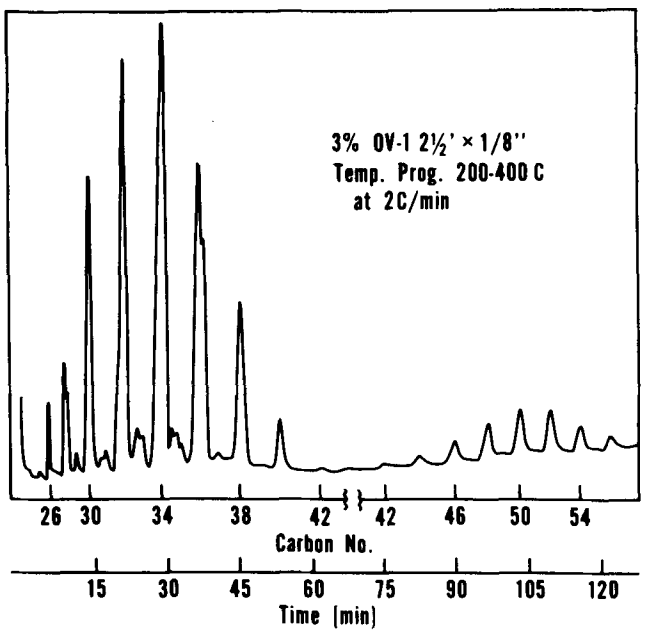



FIG. 1. Gas liquid chromatography of winterized sperm oil. Carbon numbers assigned on basis of retention times compared to jojoba-soybean oil 70:30 standard.

## EXPERIMENTAL PROCEDURES

The two winterized sperm oils investigated were Moby Dick 45NW (Werner G. Smith, Inc.) and 40.5NW Spermoil (Archer Daniels Midland Co.).

For gas liquid chromatography (GLC) of the intact oils, an F&M 810 gas chromatograph (Hewlett-Packard Co.) with a 2 1/2 ft x 1/8 in. stainless steel column packed with 3% OV-1 on 100/120 mesh Gas Chrom Q (Applied Science Lab.) was used. The injection port of the chromatograph was set at 300 C, the flame ionization detector at 350 C, and the column oven was temperature-programed from 200 to 350 C at 2 C/min. Jojoba oil (70%) plus refined soybean oil (30%) was run as a standard, and carbon numbers of sperm oil constituents were assigned by comparison of retention times with those of wax esters (8) or triglycerides in the reference mixture.

Preparative thin layer chromatography (TLC) of oil was carried out on 1 mm layers of Silica Gel G (Merck) with benzene-hexane 50:50 as the developing solvent. The plates were sprayed with ethanolic 2',7'-dichlorofluorescein, and the bands observed under UV light were scraped from the plates. The components were recovered from the silica gel with ether.

Saponification and recovery of unsaponifiable materials were performed in accordance with AOCS Tentative Method Ca 6b-53, after which the aqueous layers were collected, acidified and extracted with ether. Since hydrolysis presumably proceeds more slowly with wax esters than with triglycerides, samples were refluxed 2-3 hr instead of the recommended 1 hr. Methyl esters from intact oil, free acids and triglycerides were prepared with $BF_3$-methanol reagent (9). Alcohols were acetylated overnight in 1:1 mixtures of acetic anhydride and pyridine. Alcohol acetates were recovered by ether extraction of the acetylation mixture after addition of water. Plates spread with 1 mm layers of Silica Gel G containing 20% $AgNO_3$ were used for preparative TLC of the methyl esters and acetylated alcohols. Benzene was the developing solvent; detection and recovery of the bands were accomplished as above. From 80 to 90% of the quantities of materials applied to the plates were recovered. For both types of preparative TLC experiments, analytical-scale samples of the fractions were examined on 0.275 mm layers with appropriate absorbents and solvents. On these plates, bands were made visible by charring.

Methyl esters and alcohol acetates were analyzed in a Packard 7401 gas chromatograph (Packard Instrument Co.) equipped with flame ionization detectors and two glass columns: One, 12 ft x 1/4 in., packed with 5% LAC-2-R446 and the other, 4 ft x 1/4 in., packed with 5% Apiezon L. Peak areas were integrated electronically with a CRS-11AB/H/40-TS system (Infotronics Corp.). Equivalent chain lengths (ECL) for the components were determined from both columns. For methyl esters, saturated standards were the bases for ECL calculations (10,11) and for alcohol acetates, a similar relationship to acetylated, saturated alcohols was used, i.e., 16:0 alcohol acetate was assigned an ECL of 16.0.

TABLE I

Composition of Winterized Sperm Oil by Gas Liquid Chromatography

| Wax esters | | | Triglycerides | | |
|---|---|---|---|---|---|
| Carbon no. | Area % | Corrected %[a] | Carbon no. | Area % | Corrected %[a] |
| 24 | Trace | Trace | 40 | 0.2 | 0.5 |
| 26 | 1.1 | 0.9 | 42 | 0.5 | 1.2 |
| 27 | 0.1 | 0.1 | 44 | 0.8 | 1.9 |
| 28 | 3.3 | 2.6 | 46 | 1.7 | 4.0 |
| 29 | 0.6 | 0.5 | 48 | 2.7 | 6.4 |
| 30 | 8.2 | 6.4 | 50 | 3.1 | 7.3 |
| 31 | 1.1 | 0.9 | 52 | 2.1 | 4.9 |
| 32 | 16 | 13 | 54 | 1.4 | 3.3 |
| 33 | 2.4 | 1.9 | 56 | 1.1 | 2.6 |
| 34 | 24 | 19 | 58 | 0.3 | 0.7 |
| 35 | 2.9 | 2.3 | 60 | Trace | Trace |
| 36 | 17 | 13 | Total | 13.9 | 32.8 |
| 37 | 0.3 | 0.2 | | | |
| 38 | 6.8 | 5.2 | | | |
| 39 | Trace | Trace | | | |
| 40 | 1.8 | 1.4 | | | |
| 42 | 0.1 | 0.1 | | | |
| Total | 85.7 | 67.5 | | | |

[a]Corrected to proportions found from saponification (see text). Factors: wax ester = area % x 67/86; triglyceride = area % x 33/14.

Methoxy derivatives of monoenoid methyl esters and alcohol acetates were prepared with mercuric acetate in methanol (12) and were analyzed by gas chromatography-mass spectrometry (GC-MS) through a Packard 7401 gas chromatograph attached to a CEC 21-492-1 mass spectrometer (Dupont Instruments Corp.). For these analyses, either a 2 ft x 1/4 in. or a 6 ft x 1/4 in. glass column packed with 5% Apiezon L was used in the gas chromatograph. The ionization voltage of the mass spectrometer was 70 eV, and the source temperature was held at 200 C. The gas chromatograph was operated at various temperatures depending upon the sample. Scans were taken at the apexes of peaks and shoulders as determined from the recording of the total ionization current.

IR spectra were obtained from liquid films on NaCl disks with an Infracord 137 (Perkin-Elmer Corp.) instrument. Analyses by alkali isomerization were performed in accordance with the AOCS Official Method Cd 7-58, which uses a 6.6% KOH solution and 25 min reaction time. For quantitation, absorption at 232 nm was calculated as arising from conjugated 18:2 and that at 268 nm from conjugated 18:3.

Samples of unsaturated esters were hydrogenated in ethanol with palladium on charcoal as the catalyst.

## RESULTS

### Winterized Sperm Oil

Within the limits of sensitivity of the methods, no differences were detected between the two samples of winterized sperm oil by IR, TLC and GLC. They had the same Wijs iodine value of 86 and refractive index ($n_D^{40}$) of 1.4575. Further experiments reported in this paper were conducted only with the Moby Dick 45NW oil, a GLC of which appears in Figure 1. A series of wax esters from $C_{24}$ to $C_{42}$ emerged within the first 60 min. Integration of the peaks confirmed the immediate assumption (from inspection of the curve) that components with even carbon numbers from 28 to 40 were present in quantities close to a normal distribution curve having a mean at $C_{34}$. Partial resolution occurred within some of the peaks, most conspicuously at $C_{28}$ and $C_{36}$. Between the even-numbered peaks occurred another series of small ones due to odd-numbered chain (odd chain hereinafter) components. Partial resolution was even more pronounced in these odd series peaks. A little more than 70 min after injection, the triglycerides began to be detected. They ranged in chain lengths from $C_{42}$ to $C_{58}$ and also appeared to be arranged in a normal distribution pattern. Traces of odd chain triglycerides could be detected between some of the even-numbered peaks.

Saponification of the oil yielded 34% alcohols and 60% free acids (and no unhydrolyzed starting material detectable by TLC). The wax esters had an average molecular weight of 504 (determined from the area percentages in Table I), and the alcohols had an average molecular weight of 254 (determined from the overall composition of the alcohols given in a later section). Accordingly the percentage of wax esters in the original oil was calculated to be 504/254 x 34 = 67%. The remainder of the oil was assumed to be composed of triglycerides. Results from preparative TLC of unsaponified oil were in excellent agreement with this calculation. Of the material recovered from the plates, wax esters represented 66% and triglycerides 31%. The remaining 3% of material migrated only a short distance from the origin and was composed of free alcohols and acids.

Composition of the sperm oil by GLC is given in Table I. Peaks amounting to 86% of the total area were in the wax ester region with only 14% representing triglycerides. The corrected percentages given in Table I reflect the correction factors: % wax ester = area % by GLC x 67/86; % triglyceride = area % by GLC x 33/14. This method of correction for column and detector response assumes that each type of compound has the same response regardless of chain length. This hypothesis is probably not exactly correct, but at least the data in Table I represent a reasonable estimate of the overall composition of the sperm oil. The unhydrolyzed wax ester and triglyceride fractions, when analyzed after separation, equaled the proportions given by analysis of the intact oil.

The oil was subjected to alkali isomerization to determine how much conjugatable polyunsaturation there was. UV absorption before alkali treatment indicated apparent preformed conjugation totaling 0.9% as diene with no absorption in the conjugated triene region. After isomerization an additional 0.8% conjugated diene had been formed, and absorption equivalent to 3% of conjugated triene was observed. The figure for nonconjugated diene was in fair agreement with our analyses of the oil components, but no explanation for the appearance of $\lambda_{max}$ 268 chromophore was forthcoming since no fatty acid or alcohol moiety responsible for it was found.

TABLE II

Saturated Acids of Winterized Sperm Oil (as Methyl Esters)[a]

| ECL | | Area % by GLC | $M^+$ | Identification by GC-MS chain length |
|---|---|---|---|---|
| Apiezon L | LAC-2-R446 | | | |
| 10.0 | 10.0 | 0.4 | 186 | *n*-10 |
| 10.7 | 10.5 | 0.05 | 200 | iso-11 |
| 11.0 | 11.0 | 0.1 | 200 | *n*-11 |
| 11.4 | 11.3 | 0.05 | 214 | Me-11 |
| 11.6 | 11.5 | 0.1 | 214 | iso-12 |
| 12.0 | 12.0 | 8.5 | 214 | *n*-12 |
| 12.4 | 12.3 | 0.9 | 228 | Me-12 |
| 12.6 | 12.7 | 0.4 | 228 | iso-13 |
| 13.0 | 13.0 | 0.5 | 228 | *n*-13 |
| 13.4 | 13.3 | 0.1 | 242 | Me-13 |
| 13.6 | 13.7 | 0.4 | 242 | iso-14 |
| 14.0 | 14.0 | 26 | 242 | *n*-14 |
| 14.4 | 14.5 | 1.1 | 270 | triMe-13 |
| 14.6 | 14.7 | 1.2 | 256 | iso-15 |
| 15.0 | 15.0 | 1.8 | 256 | *n*-15 |
| 15.3 | 15.4 | 0.1 | — | triMe-14[b] |
| 15.6 | 15.7 | 0.4 | 270 | iso-16 |
| 16.0 | 16.0 | 45 | 270 | *n*-16 |
| 16.4 | 16.5 | 1.0 | 312 | tetraMe-15 |
| 16.6 | 16.7 | 1.1 | 284 | iso-17 |
| 17.0 | 17.0 | 1.2 | 284 | *n*-17 |
| 17.4 | 17.5 | 1.3 | 326 | tetraMe-16 |
| 18.0 | 18.0 | 6.5 | 298 | *n*-18 |
| 18.4 | 18.5 | 0.1 | — | tetraMe-17[b] |
| 18.6 | — | 0.1 | — | iso-19[b] |
| 19.0 | 19.0 | 0.1 | 312 | *n*-19 |
| 20.0 | 20.0 | 0.7 | 326 | *n*-20 |
| 22.0 | 22.0 | 0.1 | — | *n*-22[b] |

[a]ECL = equivalent chain length; GLC = gas liquid chromatography; GC-MS = gas chromatography-mass spectrometry.

[b]Peaks too small for reliable mass spectra; tentative identification is based on ECL values only.

## Fatty Acids

GLC of the methyl esters—whether derived from intact oil, its constituent wax esters or the triglycerides—indicated the presence of an unexpectedly wide variety of components, including some unusual ones. Obviously some separation of structural types was necessary before measurement and identification of these unusual components could be made. Esters prepared from intact oil were selected for these further investigations. TLC on silver nitrate-impregnated plates gave excellent separation between saturated (24%), monoenoic (69%) and dienoic esters (5%). No trienoic esters could be detected, even though a fraction migrating near the origin was collected. This 2% of material turned out to be composed of free fatty acids. IR of the fractions showed them to be free of *trans* unsaturation with no absorbance at 960-980 $cm^{-1}$.

TABLE III

Monoenoic Acids of Winterized Sperm Oil (as Methyl Esters)[a]

| Chain length of monoene | Area % by GLC | Relative proportions of positional isomers by GC-MS[b] | | | | |
|---|---|---|---|---|---|---|
| | | Δ5 | Δ7 | Δ9 | Δ11 | Δ13 |
| 12 | 0.9 | 100 | — | — | — | — |
| 13 | 0.1 | 100 | — | — | — | — |
| 14 | 6.9 | 20 | 10 | 70 | — | — |
| 15 | 0.3 | 40 | 40 | 20 | — | — |
| 16 | 25 | — | 80 | 20 | — | — |
| 17 | 1.5 | — | — | 100 | — | — |
| 18 | 39 | — | 7 | 86 | 7 | — |
| 19 | 0.5 | — | — | 67 | 33 | — |
| 20 | 16 | — | — | 50 | 50 | — |
| 22 | 6.6 | — | — | 17 | 66 | 17 |
| 24 | 0.4 | | | | | |
| 7-Me-16:1-6 | 1.5 | | | | | |
| Me-18:1-9 | 0.4 | | | | | |

[a]For abbreviations see Table II.

[b]Based upon relative intensities of ions from cleavage of methoxy derivatives (see text).

The saturated esters were composed of at least three homologous series ranging from $C_{10}$ to $C_{22}$ (Table II). The most prominent series was that of the normal esters. A second group had ECL values 0.4 units greater than the normal esters eluting immediately before them from Apiezon L columns, and the third series had ECL values (Apiezon L) 0.6 units more than the straight chain compounds. Compounds in the second series (+0.4 ECL units) with less than 14 carbons appeared to be substituted with one methyl group. Mass spectra and ECL values indicate that the branching occurs at the third carbon atom (13,14). Beyond $C_{14}$, members of this +0.4 ECL series gave mass spectra similar to those of esters of the isoprenoid acids studied by Douglas et al. (15), and the ECL increment is actually 1.4 units more than the straight chain backbone without the nonterminal C-methyl groups (15). Sano has identified three isoprenoid acids in whale oils: 4,8,12-trimethyltridecanoic; 2,6,10,14-tetramethyltetradecanoic; and 3,7,11,15-tetramethylhexadecanoic (16-18).

The highest homologous series (+0.6 ECL units) was established as isoesters from their ECL values (14) and mass spectra.

The monoenoic acids of sperm oil were arrayed in chain lengths from $C_{12}$ to $C_{24}$. Their composition is given in Table III. Mass spectra of the methoxy derivatives adequately defined the locations of the double bonds in major components (12,19).

In straight chain unsaturated esters, addition of methanol occurs equally on either side of isolated double bonds located four or more carbons from the carbomethoxy group (12). Consequently two methoxy esters are produced from each monoene. During MS, cleavages adjacent to the carbon atoms bearing methoxyl groups produce intense ions, typically four per parent monoene. Relative abun-

TABLE IV

Saturated Alcohols of Winterized Sperm Oil (as Acetates)[a]

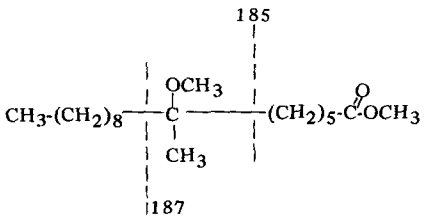
| ECL | | Area % | | Identification |
|---|---|---|---|---|
| Apiezon L | LAC-2-R446 | by GLC | $(M-60)^+$ | by GC-MS chain length |
| 14.0 | 14.0 | 8.4 | 196 | *n*-14 |
| 14.6 | 14.5 | 0.5 | 210 | iso-15 |
| 15.0 | 15.0 | 2.3 | 210 | *n*-15 |
| 15.6 | 15.5 | 0.5 | 224 | iso-16 |
| 16.0 | 16.0 | 70 | 224 | *n*-16 |
| 16.6 | 16.5 | 1.9 | 238 | iso-17 |
| 17.0 | 17.0 | 2.0 | 238 | *n*-17 |
| 17.6 | 17.5 | 1.1 | 252 | iso-18 |
| 18.0 | 18.0 | 11 | 252 | *n*-18 |
| 18.6 | 18.5 | 0.2 | 266 | iso-19 |
| 19.0 | 19.0 | 0.2 | 266 | *n*-19 |
| 20.0 | 20.0 | 0.2 | --- | *n*-20[b] |

[a]For abbreviations see Table II.

[b]Peak too small for reliable mass spectrum; tentative identification based on ECL values only.

dance of positional isomers given in Table III was calculated from the averages of the intensities of these four ions.

Two branched chain monoenes were shown to be present. One of these, which as the underivatized methyl ester had ECL values similar to those found by Sano (20) for 7-methyl-6-hexadecenoate, gave an atypical mass spectrum after methoxylation. This spectrum had only two intense ions located at m/e 187 and 185. These would result if the methoxyl group had preferentially added to form only one isomer:




Virtually exclusive addition of the analogous hydroxyl ion to the branched carbon was shown by Brown and Geoghegan (21) in reactions with 2-methyl-1-butene and 2-methyl-2-butene. The mass spectrum and ECL data strongly suggest that we have encountered the same substituted $C_{17}$ olefinic acid described earlier in fin whale blubber (20). However the double bond position cannot be unequivocally established since 7-methyl-7-hexadecenoic acid would give an identical mass spectrum (after derivitization) and has also been found in fish oils (22).

The branched chain $C_{19}$ monoene yielded the expected four ions from its methoxy derivatives at m/e = 229, 215, 171 and 157. These ions indicate that this ester had a 9,10 double bond (ions 157 and 171 [12]) and that the branch was located between the carboxyl group and the double bond (ions 215 and 229). GLC results from a hydrogenated sample of the monoenes were in quantitative agreement with those from the unhydrogenated sample.

The diene fraction contained 8% of 14:2, 12% of 16:2, 31% of 18:2, 6% of 20:2 and traces of 22:2 and odd chain dienes. The remainder appeared to be monoenes carried over from preparative TLC. This fraction, containing ca. 60% dienes, amounted to 5% of the total material recovered from preparativw TLC. Since fatty acids make up ca. 60% intact sperm oil, the diene proportion found by GLC is (0.60 x 0.05 x 0.60) x 100 or ca. 1.8% of the original winterized oil. This figure is in approximate agreement with that found by alkali isomerization (0.8%). GLC and GC-MS analyses failed to reveal any components more highly unsaturated than dienes.

### Fatty Alcohols

Preparative TLC of the acetylated alcohols gave 40% saturated, 56% monoenes, 3% dienes and 1% at the origin. GLC of the fractions showed that the material collected from the diene region was actually composed of a mixture of monoenes and dienes. Material collected near the origin was made up of free alcohols. Again, no *trans* absorption was detected in the IR spectra of the fractions.

The alcohols of sperm oil are not nearly so diverse as the fatty acids. Composition of the saturated alcohol acetates is given in Table IV. Traces of $C_{10}$, $C_{12}$ and $C_{22}$ alcohols were also indicated from ECL data. During mass spectroscopy, the acetate group is easily lost (23) so that molecular

TABLE V

Monoenoid Alcohols of Sperm Oil (as Acetates)[a]

| Chain length of monoene | Area % by GLC | Relative proportion from GC-MS of winterized oil[b] | | | | Positional isomers previously reported for refined oil[c] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Δ7 | Δ9 | Δ11 | Δ13 | Δ5 | Δ7 | Δ9 | Δ11 | Δ13 |
| 14 | 0.1 | | | | | 48 | 40 | 12 | --- | --- |
| 15 | 0.1 | | | | | | | | | |
| 16 | 11 | 25 | 75 | --- | --- | --- | 71 | 24 | 5 | --- |
| 17 | 2.4 | --- | 100 | --- | --- | | | | | |
| 18 | 76 | 8 | 84 | 8 | --- | --- | 4 | 84 | 9 | 3 |
| 19 | 1.1 | --- | 67 | 33 | --- | | | | | |
| 20 | 6.7 | --- | 20 | 75 | 5 | --- | --- | 3 | 73 | 24 |
| 7-Me-16:1-6 | 1.0 | | | | | | | | | |

[a]For abbreviations see Table II.

[b]Based upon relative intensities of ions from cleavage of methoxy derivatives (see text).

[c]Hamilton et al. (25).

ions are not intense, but the ions at M-60 are prominent (24) and serve to indicate molecular weights.

In Table V, the monoenoid alcohols identified by GC-MS are listed along with data previously reported (25). A branched olefinic alcohol, analogous to the 7-methyl-$C_{16}$ fatty acid, was also found. Unlike the saturated alcohol acetates, the ions at M-60 for the methoxy derivatives are much less prominent than the intense ions from each of the two cleavage sites alpha to the methoxyl group. As in the saturates, traces of 10:1, 12:1 and 22:1 alcohols were indicated by ECL. GLC of the hydrogenated sample confirmed the identities listed in Table V.

Of the diene fraction making up 3% of the alcohols, ca. 35% was 18:2 and ca. 17% was 20:2. Small amounts (ca. 1%) of the other dienes were also suggested. Calculations from these percentages and the 34% yield of alcohols from saponification give 0.5% dienoic alcohols in the sperm oil. Again no more highly unsaturated species could be found.

## DISCUSSION

The range and distribution of constituent carbon numbers within the wax esters and triglycerides may contribute to the usefulness of winterized sperm oil in the manufacture of lubricant products. Conceivably, replacements having homologous constituents with a normal distribution of carbon numbers might perform most like it. With respect to sperm oil triglycerides, this normal distribution is not to be confused with random intraglyceride distribution of acyl groups. Barr et al. (4) found significant deviation from the latter.

Less obviously, the bell-shaped patterns in Figure 1 also do not necessarily mean random combination of all moieties present, i.e., of acids with alcohols in wax esters and of the three acids (without regard to positional preferences) in triglycerides. For example, a disproportionately large amount of a given short chain alcohol might be offset by preferential combination of it with longer chain acids. However the concentrations we found of various alcohol and acid moieties in winterized sperm oil happened to be such that calculations based on assumed random combination gave compositions fairly close to those in Table I. Agreement was complete at the mean wax ester ($C_{34}$) and within 1% for the most abundant triglycerides ($C_{50}$ and $C_{52}$). Found peak areas were generally 1 or 2% more than calculated amounts for shorter chain components and vice versa for longer ones, perhaps reflecting a decrease in GLC response with increased molecular weight. Incidentally plotting the data of Challinor et al. (3) for sperm head wax esters gives a pattern truncated at $C_{38}$ but otherwise qualitatively similar to the one for wax esters in Figure 1.

Sulfurization is employed in the manufacture of commercial extreme-pressure lubricant additives from winterized sperm oil (7). Although the reaction is not completely understood, mechanisms proposed for it involve addition of sulfur at unsaturated sites to produce polysulfides (26). Consequently the high proportion of monoenes (69% of acid and 56% of alcohol moieties) makes most of the oil molecules susceptible to this modification. Increased structural diversity in the derivatized material may be a desirable consequence of the variation in double bond position (Tables III and V). With further regard to double bond position, the differences shown in Table V between our results and those of Hamilton et al. (25) warrant comment. We believe these discrepancies reflect compositional differences in the sperm oil samples studied. Hamilton and his coworkers (25) referred to their oil as refined but did not call it winterized. The winterization process to which our samples had been subjected may very well have preferentially removed wax esters with double bonds in the alcohol moiety closer to the ester function.

Presumably unidentified chromophores present in trace amounts contributed to the apparent polyunsaturation in winterized sperm oil as determined by alkali isomerization and UV measurement. The level (<2.3% total) as measured by GLC is probably more accurate. Larger quantities of polyunsaturated moieties would be expected to affect unfavorably the stability of the oil and its behavior during and after the sulfurization process. Although only those monoenes with double bonds located an odd number of carbon atoms from the ester group are listed in Tables III and V, monoenes with the unsaturation in an even position cannot be distinguished if both adjacent odd position isomers are present, e.g., 16:1-7 and 16:1-9 give 8- and 9-methoxy derivatives, the same derivatives formed from 16:1-8.

By decreasing the complexity of the methyl ester and alcohol acetate mixtures through preliminary separation into saturated, monoenoic and dienoic fractions and by applying GC-MS to minor components in these fractions, we investigated branched chain components of sperm oil in more detail than ever before. The 7% plus of total odd chain components (both branched and straight) may be important in determining fluid properties of sperm oil. The known lower melting points of branched and odd chain fatty acids, as compared to their straight even chain counterparts (27), support this speculation.

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