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The chromatographic analysis of spermaceti

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The alkyl esters of spermaceti can be analysed directly by high temperature gas chromatography on a 10% Silicone Elastomer E301 column following the injection of the whole wax. The validity of the technique was established by initial chromatographic work on the isolated alkyl ester fraction and its saponification products. In three commercial wax samples, four homologues C_{28} (hexadecyl dodecanoate), C_{30} (hexadecyl tetradecanoate), C_{32} (hexadecyl hexadecanoate) and C_{34} (hexadecyl octadecanoate), accounted for 86–89% of the total alkyl esters. Variation in the ester homologue content is produced only by variations in the fatty acid homologue content.

THE major constituents of spermaceti have long been known to be long chain alkyl esters comprised of primary alcohols and saturated fatty acids. However, there is no general agreement about the exact composition of the ester fraction. Most authors report that the fraction is chiefly cetyl palmitate $C_{15}H_{31}COOC_{16}H_{33}$ (Pratt & Youngken, 1956; Trease, 1966; Claus & Tyler, 1965; Warth, 1956). Others have reported the esters to be predominantly a mixture of cetyl palmitate and cetyl myristate in approximately equal proportions (Wallis, 1962; BPC, 1963).

This paper describes further work on the alkyl ester constituents of spermaceti using thin-layer, preparative-layer and gas chromatographic methods for analysis.

Experimental

Melting points were determined on a Kofler block. Infrared absorption spectra were obtained with a Unicam SP 200 infrared spectrophotometer; the samples were examined in potassium chloride discs (1–2 mg in 200 mg).

THIN-LAYER CHROMATOGRAPHY

The qualitative composition of the wax was determined by the methods of Holloway & Challen (1966). Wax constituents are fractionated into classes of compound, not into individual compounds, by thin-layer chromatography (Malins & Mangold, 1960). The alkyl ester content of the wax was determined by preparative-layer chromatography on 1·5 mm layers of Kieselgel H using the solvent system carbon tetrachloride/chloroform: 95/5 v/v (Holloway 1967). The alkyl ester fraction isolated was identified by infrared spectroscopy, the ferric hydroxamate colour test (Goddu, Leblanc & Wright, 1955) and further thin-layer tests (Holloway & Challen, 1966).

GAS CHROMATOGRAPHY

A Pye Series 104 gas chromatograph fitted with a flame ionization detector, and a −0·1 to + 1·0 mV Honeywell-Brown Electronik recorder were used. The chromatographic columns were of stainless steel 4 mm in bore and 5 ft in length, packed with 100–120 mesh Chromosorb W coated

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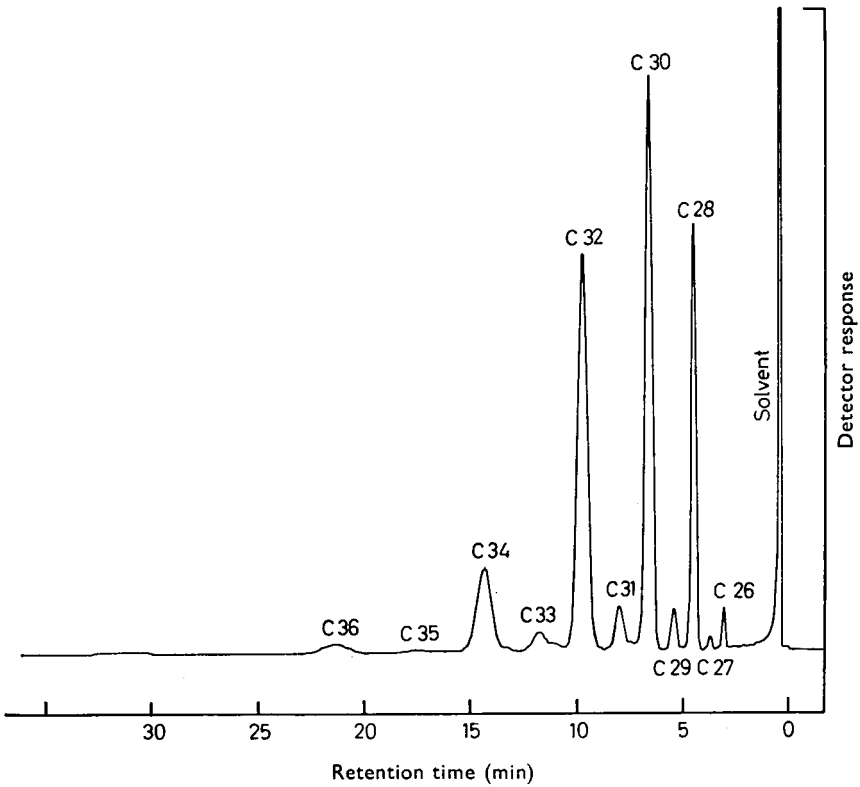

FIG. 1. Gas chromatogram of the analysis of the alkyl ester fraction from spermaceti in Sample 2.

with 10% w/w Silicone Elastomer E301 (ICI Ltd). The carrier gas was nitrogen, flow rate 40 ml/min (18 lb/inch^2), and the detector flow rates were hydrogen 40 ml/min (15 lb/inch^2) and air 420 ml/min (20 lb/inch^2). Chromatograms were run isothermally using a chart speed of 20 inches/hr. All samples were injected as 2·0% solutions in chloroform or benzene; the injection volume being in the range 0·2–1 μl. Before use the columns were conditioned for at least 24 hr with the oven temperature 25° above the required operating temperature and using full carrier gas flow.

Spermaceti and its alkyl ester fraction were analysed at 300°. Peaks were identified by comparison of retention times with those of reference samples of alkyl esters (C_{26}, C_{28}, C_{30}, C_{32}, C_{34} and C_{40}) prepared by the method of Kaufmann & Pollerberg (1962). Saponification of the alkyl ester fraction and the recovery of the resultant alcohols and acids, were according to Mazliak (1963). The primary alcohols and fatty acids obtained were analysed isothermally at 200°. Primary alcohols were chromatographed as their acetate derivatives (Holloway, 1967) and identified by comparison with reference primary alcohol acetates (C_{12}, C_{14}, C_{16}, C_{18} and C_{20}). Fatty acids were chromatographed as their methyl

ester derivatives (Metcalfe & Schmitz, 1961) and identified by comparison with reference fatty acid methyl esters (C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}, C_{20},C_{22} and C_{24}). Further identification of alkyl ester, primary alcohol acetates and fatty acid methyl esters was achieved by plotting log retention time (from the solvent peak) against carbon number (James & Martin, 1952). Completeness of elution of all chromatograms was checked by running the fractions at higher temperatures and for longer times, which would indicate the presence of any higher homologues.

The quantitative composition of the fractions was determined from the chart by computation from the peak areas using the triangulation method. The area obtained is 97% of the actual area (Condal-Bosch, 1964). Each constituent of a fraction was expressed as the ratio of its peak area to the summation of the areas of all peaks. Mean values were calculated from five replicate determinations.

Results and discussion

The major constituent of spermaceti by thin-layer chromatography corresponded in Rf value with an alkyl ester. Trace amounts of other constituents corresponding to alkane, secondary alcohol and fatty acid were also detected. No sterols or sterol esters, reported to be present in spermaceti by Warth (1956), were detected. The three commercial spermaceti samples gave identical thin-layer patterns. Adulterants of spermaceti reported by Wallis (1962) and BPC 1963 could be also readily detected by the presence of anomalous spots in the thin-layer chromatographic patterns. Paraffin wax moved above, while stearin and tallow (predominantly triglycerides) and stearic acid moved well below, the major alkyl ester spot of spermaceti.

The fraction which corresponded in Rf value with an alkyl ester was isolated by preparative layer chromatography and gave a strong violet colour with the ferric hydroxamate test. Its infrared spectrum showed strong bands at 2851–2890 cm^{-1} and medium bands at 1465–1470 cm^{-1} due to CH_3 and $-CH_2-$ absorption and medium bands at 720–729 cm^{-1} due to $-(CH_2)_4-$ absorption. Strong bands at 1730–1735 cm^{-1} and 1180 cm^{-1} were also present, due to $>C=0$ absorption. These absorptions are typical of a long-chain alkyl ester and were identical with a reference spectrum of octadecyl docosanoate. The melting points of the three fractions isolated from three samples of spermaceti were: for Sample 1, 50–51°, for Sample 2, 49–50° and for Sample 3, 48·5–49·0° (reported melting points for cetyl palmitate range from 51·5–53°). The alkyl ester content of the three waxes, as determined by preparative-layer chromatography of known weights of wax, was for Sample 1, 92·8%, for Sample 2, 95·0% and for Sample 3, 94·2%. These are the mean values based on 3 separate determinations. The figures are in agreement with Warth (1956) and Cole & Brown (1960) but higher than the 80% quoted by Wallis (1962).

Well resolved symmetrical peaks suitable for quantitative evaluation were obtained from all the fractions examined by gas chromatography.

The direct analysis of alkyl esters derived from natural waxes has only been briefly reported before by Radler (1965) and Ludwig (1966). Every peak in the three alkyl ester fractions examined corresponded in retention time with that of a n-alkyl ester. A representative gas chromatogram of the analysis of the alkyl ester fraction from spermaceti Sample 2 is shown in Fig. 1. The percentage composition of the alkyl ester fractions is recorded in Table 1. Homologues with an even number of carbon atoms predominated. The principal homologues were C_{28}, C_{30}, C_{32} and C_{34} comprising 88·7% of the total alkyl esters of sample 1, 88·8% of Sample 2 and 86·6% of Sample 3. However, the major homologues were C_{30} and C_{32}: 68·2% of Sample 1, 62·5% of Sample 2, and 59·4% of Sample 3.

TABLE 1. COMPOSITION (%) OF THE ALKYL ESTER FRACTIONS FROM THREE COMMERCIAL SPERMACETI SAMPLES

Carbon number	Sample 1*	Sample 2**	Sample 3***
<26	Trace	Trace	Trace
26		1·5	1·9
27		0·6	1·0
28	3·0	15·3	18·9
29	0·8	2·1	2·4
30	26·0	31·8	36·4
31	3·2	2·8	2·5
32	42·2	30·7	23·0
33	3·0	1·6	1·9
34	17·5	11·0	8·3
35	1·2	0·6	0·7
36	3·1	2·1	3·0
37	Trace	—	—
38	Trace	Trace	Trace
>38	—	—	—

* Sample 1—Museum sample (Pharmacognosy Dept.) M.p. 46·0–47·0°C.
** Sample 2—Supplied Brohme & Schimmer Ltd. (1965) M.p. 47·0–47·5°C.
*** Sample 3—Supplied Brohme & Schimmer Ltd. (1966) M.p. 46·0–47·0°C.

TABLE 2. COMPOSITION (%) OF THE FATTY ACIDS DERIVED FROM THE SAPONIFICATION OF THE ALKYL ESTER FRACTIONS FROM THREE COMMERCIAL SPERMACETI SAMPLES

Carbon number	Sample 1	Sample 2	Sample 3
<10	Trace	Trace	Trace
10		1·8	2·0
11		0·5	1·1
12	2·3	14·1	20·0
13	1·0	2·0	2·4
14	26·0	32·0	35·0
15	4·1	2·6	2·3
16	40·3	30·0	23·9
17	3·5	2·1	1·6
18	18·0	11·4	9·4
19	1·9	0·8	0·3
20	2·9	2·7	2·0
21	—	—	—
22	Trace	Trace	Trace
>22	—	—	—

The direct analysis of the esters, however, gives only the carbon number of the homologues but no information of the acids or alcohols which constitute the esters. The latter information was obtained from the gas

chromatographic examination of the saponification products of the alkyl ester fractions. The alcohol portion of the three esters was found to be nearly pure hexadecanol containing trace amounts of tetradecanol and dodecanol. The alkyl esters of spermaceti must therefore be predominantly hexadecyl esters. Wellendorf (1963) found that the alcohol fraction from one sample of spermaceti comprised 80% hexadecanol, with tetradecanol and octadecanol comprising the remainder. The acid portion of the esters showed a much wider distribution of homologues which satisfactorily accounted for the homologue variation of the alkyl esters (Table 2). The percentage composition of the fatty acids, although of different chain length, was similar to the alkyl esters (compare Tables 1 and 2). The principal alkyl esters of spermaceti are therefore C_{28} hexadecyl dodecanoate (cetyl laurate), C_{38} hexadecyl tetradecanoate (cetyl myristate), C_{32} hexadecyl hexadecanoate (cetyl palmitate) and C_{34} hexadecyl octadecanoate (cetyl stearate). The textbook descriptions of spermaceti as being chiefly cetyl palmitate are obviously inadequate. The description of spermaceti as being chiefly cetyl palmitate and cetyl myristate in approximately equal propotions is substantially correct. However, the present experiments have shown that a more accurate description of spermaceti would be a mixture of hexadecyl esters of fatty acids between C_{26} and C_{38} with hexadecyl dodecanoate, hexadecyl tetradecanoate, hexadecyl hexadecanoate and hexadecyl octadecanoate comprising at least 85% of the total esters.

Further experiments showed that the alkyl ester consituents of spermaceti can also be analysed directly by gas chromatography following the injection of a solution of the intact wax. This procedure provides a rapid qualitative and quantitative evaluation of wax samples ideally suited for routine screening.

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