Analysis of the Monoethylenic Fatty Acids of Rapeseed Oil by Open Tubular Gas Chromatography

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Abstract

A sample of rapeseed oil of known composition was analyzed by gas chromatography using open tubular columns and two types of polyester coating. The results confirm the previous analysis in most respects and clearly illustrate the possibility of using this technique to detect and determine isomeric forms of monoethylenic fatty acids. Some hitherto unreported trace components have been provisionally identified in rapeseed oil by linear log plots.

Introduction

THE USE OF OPEN TUBULAR (capillary or Golay) gas chromatographic columns (1,2) in fat chemistry has been largely confined to studies on hydrogenated fatty acids or other materials where prototropic reactions result in the formation of multiple isomers of both monoethylenic and polyethylenic fatty acids (3,4). The advantages of these columns in separating and identifying isomeric monoethylenic fatty acids of "normal" structures (differing in double bond position by two carbon atoms) in naturally occurring lipids have received little attention (5,6), although such separations are seldom achieved with packed columns (7). The probable reason for this limited interest is that open tubular columns can rarely be utilized successfully in apparatus designed primarily for packed columns (8). The examination of rapeseed oil reported herein was carried out to explore the use of the esters of this oil as "secondary" gas-liquid chromatographic standards in studies on the distribution of monoethylenic fatty acid isomers in marine oils. An oil previously examined in detail by conventional means (9) was obtained to permit a comparative study, through gas chromatography alone, of the overall composition and of the distribution of isomeric monoethylenic fatty acids.

Experimental

A Perkin-Elmer Model 226 gas chromatograph was operated isothermally at 170C with an injection port temperature of 260C, helium carrier gas at 40 psig input, and a No. 2 stream splitter in the injection system. Output was recorded on a Honeywell Electronik 16 (−0.05 to +1.05 mv) recorder fitted with a Model 227-S Disc Instruments, Inc., integrator. Peak areas were corrected to give weight % composition (10). Temperature programming over the range 140–170C at 0.5C/min was also employed with other operating conditions as above.

Columns were of the open tubular type, 150 ft in length and 0.01 in. internal diameter. These were supplied by Perkin-Elmer with respective coatings of butanediol-succinate (BDS) polyester and diethylene glycolsuccinate (DEGS) polyester. The columns were stabilized as recommended by the manufacturer and each used daily under the above operating conditions for approximately one month prior to carrying out the analyses reported in this paper.

Rapeseed oil (Wijs iodine value 100.5) was generously supplied by D. F. Kuemmel, The Procter and Gamble Co., Miami Valley Laboratories, Cincinnati, Ohio. After saponification and removal of nonsaponifiable materials the recovered fatty acids were esterified with boron trifluoride-methanol (11). The iodine value of the esters by semimicro hydrogenation (12) was 97.2.

Results

Provisional Identifications

The gas chromtaograms obtained with both BDS and DEGS columns showed (Fig. 1) significant separations of two isomeric monoethylenic fatty acids in the C_{16}, C_{18}, C_{20} chain lengths, but only one 24:1 acid. The major fatty acid isomers in this particular oil sample had been established by degradation (9) as the ω9 type,[1] including 24:1, excepting in the C_{16} acids where only 16:1ω7 was assumed. Minor isomers were all expected (9) to be of the ω7 type. Plots of relative retention time against fatty acid carbon chain length gave parallel straight lines for the ω9 and ω7 isomers for all even chain lengths. This procedure provided evidence for an ω9 structure in the minor C_{16} isomer immediately preceding the predominant isomer (cf 13). In addition the peak corresponding to a 17:1 acid fell between the two lines, indicating correctness of the assumption (9) that this would be 17:1ω8 as reported from other sources (13,14). A baseline irregularity, but no definite peaks, was observed in the predicted positions for 19:1 acids.

The linear log plots of the diethylenic fatty acids were facilitated by the presence of 18:2ω6, 20:2ω6 and 22:2ω6 acids (9). Extrapolation of this line indicated the presence of a small amount of 16:2ω6 in analyses on both polyesters. Similarly a parallel line through the point for 18:3ω3 from the DEGS analysis passed through points for trace peaks corresponding to 16:3ω3, 20:3ω3, 20:3ω3. The BDS analysis of the ester sample was carried out after several weeks and of the minor linolenic type acids only the 16:3ω3 and 20:3ω3 peaks were definitely observed, with the possibility of 22:3ω3 peak in the position predicted by the log plot. This difference could be ascribed to oxidation of the sample (see below). A different detailed study on rapeseed oil lists a 20:3 acid as a trace component (15).

On the BDS chromatogram the 22:0 and 24:0 acids could be readily identified and were completely separated from the 22:1 and 24:1 acids. On the DEGS chromatogram these peaks merged into the corresponding monoethylenic fatty acids. On both chromatograms a peak for 17:0 was present, but 19:0 was partially masked in the tailing of the major 18:2ω6 peak, and might not have been recognized if the position had not been indicated by the log plot.

Quantitation

The degree of separation of the 18:1, 20:1 and 22:1 isomeric pairs might be expected to depend on the sample load on the column. Injection of samples

[1] Notation for chain length:number of double bonds and position of ultimate double bond relative to terminal methyl group.

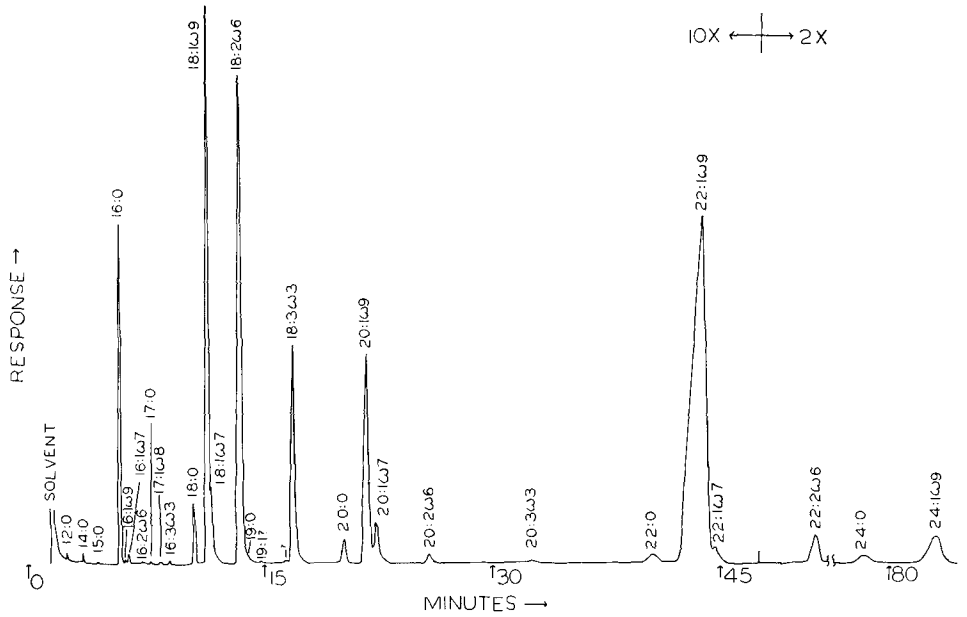

FIG. 1. Gas-liquid chromatography of rapeseed oil methyl esters on an open tubular column. BDS polyester, 150 ft. × 0.01 in. I.D., temperature 170C, carrier gas helium at 40 psig. Attenuations as marked at top.

ranging from 0.0003 to 0.001 μl indicated that this view was correct. However, at all loads the resolution of the two 20:1 isomers was markedly superior to that of the 18:1 and 22:1 isomers (Fig. 1). This suggested that the ratio of the two 20:1 isomers must differ from those of the other chain lengths. The isomer ratios reported (as approximations) from oxidative fission studies (9) are respectively, for C_{18}, C_{20} and C_{22}:- 14/1, 13/1 and 44/1. The quantitation of the incompletely resolved 18:1 and 22:1 peak pairs in the isothermal chromatograms from these open tubular column analyses was not possible on a precise basis, but some correction could be made to the peak height of the minor isomer by subtracting the tailing of the corresponding saturated acid run on the same column at the same sample load. The proportions of isomers based on adjusted peak height ratios for the C_{18} and C_{22} acids were respectively 16/1 and 37/1, nearly the same as those previously reported. The C_{20} isomers were sufficiently separated to obtain area proportions, which gave an isomer ratio of 6/1, as compared to the previous report of 13/1. Thus it is evident that where peak separation is inadequate for quantitation by areas, the ratios of peak heights can give an approximate figure for the proportions of isomers when suitably corrected for tailing of the first component. On the columns employed in the present study under the operating conditions used it is probable that a ratio of major to minor isomers of not more than 5/1 would be necessary to obtain complete separation of two isomers where the major isomer is of the ω9 type and the minor of the ω7 type. Superior resolution could be obtained when this ratio is small by reducing the sample size, since the minor isomer could still be readily seen and estimated under these conditions. When the minor isomer precedes the major, quantitation by area should be possible in most cases.

The use of temperature programming improved the resolution of isomers of common chain length by about 50%. The quantitation of the minor isomers then gave substantially the same results as isothermal operation. The baseline drift (BDS column) introduced errors in integrator area determinations which to some extent offset the improved separation. This would not necessarily apply in two-column operation where baseline drift is compensated, or if a baseline drift compensator were used.

The quantitative operation of the injection splitter, open tubular column and flame ionization detector (1) was supported by an analysis of NIH mixture "F" (16) which gave satisfactory weight % correlation of the C_{14}-C_{24} even chain acids after correction for flame ionization response (10). The overall results of the rapeseed oil ester analysis agree reasonably well with those of Kuemmel (Table I), although sample oxidation gave slightly low figures for the per cent polyunsaturated acids in the BDS analysis. The oxidation of the sample was confirmed by the presence of peaks for volatile oxidation products in the BDS chromatogram and by the cloudy appearance of the solution of esters dissolved in hexane. The iodine value calculated from the DEGS analysis (corrected for 22:0 and 24:0 from the BDS data) was 98.6 as compared to 97.2 for the experimental hydrogenation iodine value. The totals of chain lengths in the hydrogenated sample were quantitatively satisfactory. Unidentified materials comprised less than 0.10% of the net chromatographic response and consisted principally of traces of material in the 19:1 region and a minor component observed in both analyses immediately preceding the 18:3ω3 peak (Fig. 1).

Discussion

Prior to carrying out analyses reported in this paper the columns had each been employed daily for a month in various marine oil analyses. In that period typical retention times had shortened about 20%, with a detectable loss in resolving power for monoethylenic fatty acid isomers. Subsequent to the studies reported herein a new BDS column was obtained from the manufacturer. The new column operated under identical conditions gave retention times for the same materials nearly twice those of the BDS column employed in the rapeseed oil analyses and gave markedly improved resolution of isomeric monoethylenic fatty acids, as well as exhibiting greater stability in continuous operation.

The properties of open tubular columns coated with

polyesters are not very reproducible and may be modified even by the nature of the solvent used to dissolve the liquid phase (17). This possibly accounts for the observation that the BDS and DEGS columns used in the present study had nearly identical retention times and polarities. In packed columns the latter property may be modified by the percent substrate, type of support and operating conditions (18), but there are as yet inadequate data from open tubular columns to examine polyester polarity in detail. The identities of the BDS and DEGD polyesters were confirmed by the behavior of the longer chain saturated fatty acids. In the DEGS analyses the poor separation of these acids from the monoethylenic acids in the C_{22} and C_{24} chain lengths reflects the convergence of the two linear log plot lines with increasing chain length. This behavior is noticeable in the more polar polyesters at lower operating temperatures (19,20) but in analyses on the less polar BDS the two lines are normally nearly parallel. In the BDS linear log plot the polyethylenic fatty acid lines were apparently parallel to the lines of the two monoethylenic fatty acid systems. In the DEGS log plot the polyethylenic fatty acid lines definitely converged slightly with the monoethylenic lines as the chain length increased. This may also be a chemical property of the normally more polar polyesters as indicated by the retention time data tabulated by Hofstetter et al. (21).

Of the two polyesters employed the BDS is preferred since the 22:0 and 24:0 peaks are well separated from the corresponding monoethylenic fatty acids. There is also an advantage in having the polyethylenic fatty acid lines in a linear log plot virtually parallel to the monoethylenic fatty acid lines (19,20).

The separation factors (i.e., a longer retention time divided by a shorter retention time) for the ω9 and ω7 series isomers on both BDS and DEGS columns were consistent for all chain lengths at about 1.036. Panos (6) reported data for separations of some bacterial monoethylenic fatty acids of the C_{14}, C_{16} and C_{18} chain lengths on a Carbowax open tubular column which give a corresponding figure of 1.034 for these separations and a figure of 1.041 in the case of the ω7 and ω5 isomers. The larger figure in the latter case may be expected as the numerical values of the end carbon chains (ω) in the isomers diminish (18). The separation factor obtained on the Carbowax column for fatty acids of the ω9 and ω7 types may have been similar to the BDS and DEGS results because the separations in this instance are primarily based on the physical properties of the isomers (18–20, cf 22) It should be noted that the type of log plot originally used by Panos (6) to illustrate the structures of the bacterial fatty acids may equally well be redrawn to join points of common end carbon chain (ω), thus permitting the joining of three points in the ω9 series, three points in the ω7 series, and two points in the ω5 series with parallel straight lines (19).

Open tubular columns of higher efficiencies with Apiezon L as a substrate (5) show markedly improved separations, and as mentioned above the separations observed with the present type of polyester columns could be improved. It is reported that elaidic acid may be observed as an artifact in olive oil fatty acid ester analyses if columns have been inadequately conditioned (5). The analysis of animal isomeric monoethylenic fatty acids may be less feasible if certain abnormal isomers (23) in which the double bond position is "even" with respect to the carboxyl group are prominent. However, this would depend on the particular double bond positions, and on the relative proportions. The 18:1ω9 *trans* isomer falls before the *cis* isomer on polar columns (3) and after it on Apeizon columns (3,5,24,25). This appears to be a generally applicable rule, since on polar nitrile silicone polymers *trans* 18:1ω9 precedes *cis* 18:1ω9 (26). On Apiezon columns *trans* isomers could therefore be confused with *cis* isomers with double bonds closer to the terminal methyl group (27). The latter have longer retention times, increasing nonlinearly as the ω values decrease on both polar and Apiezon packed columns (18,25,28). This is also true on Apiezon open tubular columns (3,28), but 18:1ω12 has been reported (5) to have a longer retention time than 18:1ω9, or to fall immediately before 18:1ω9 (29), in Apiezon open tubular analyses. Possibly 18:1ω12 has been confused with 18:1ω7 (cf 5, 29–32). It is known that on BDS open tubular columns 18:1ω12 falls before 18:1ω9 with very little separation (33), and an examination of isomeric methyl nonynoates (34) suggests that only unsaturation in the 2–3 and 3–4 positions relative to the carboxyl group will interact with the latter to give increased retention times on both polar and Apiezon open tubular columns.

Caution must therefore be observed in interpreting peaks with retention times longer than those of the common isomers with centrally located double bonds, although in the rapeseed oil examined in the present analysis there were no *trans* isomers in the original material or in esters prepared by interesterification and isolated by mercury derivative chromatography (9). The successful application of open tubular gas chromatography to isomer studies, as in many applications of gas chromatography, requires some knowledge of the material under study and does not constitute definite proof of identification.

TABLE I

Weight Percent Composition of Rapeseed Methyl Esters as Determined by Open Tubular Gas Chromatography on Two Polyesters

Fatty acid[a]	Analysis on BDS polyester[b]	Analysis on DEGS polyester	Identical oil analysis by Kuemmel (9)[c]
12:0	0.02	0.03	0.02
14:0	0.11	0.08	0.06
15:0	0.01	0.01	NM
16:0	4.0	4.2	3.6
16:1ω9	0.06	0.07	}
16:1ω7	0.13	0.14	} 0.21[d]
16:2ω6	0.03	0.04	NM
16:3ω3	0.05	0.07	NM
17:0	0.03	0.02	0.05
17:1ω8	0.02	0.01	NM
18:0	1.27	0.94	1.37
18:1ω9	16.4	15.5	14.6
18:1ω7	1.0	0.8	1.1
18:2ω6	12.7	13.7	14.0[e]
18:3ω3	5.3	6.9	6.4[e]
19:0	0.06	0.02	0.01
19:1?	0.03	?	NM
20:0	0.87	0.90	0.84
20:1ω9	9.0	8.6	9.2
20:1ω7	1.4	1.9	0.7
20:2ω6	0.32	0.55	0.64
20:3ω3	?	0.13	NM
22:0	0.65	}	0.72
22:1ω9	44.4	} 42.5	43.3
22:1ω7	1.2	1.3	1.0
22:2ω6	0.17	0.48	0.64
22:3ω3	?	0.14	NM
24:0	0.16	}	0.09
24:1ω9	0.64	} 0.81	0.42
Σ Saturated	7.2	7.0	6.8
Σ Monoethylenic	74.3	71.0	70.5
Σ Polyethylenic	18.5	22.0	22.7

[a] Notation for chain length:number of double bonds and position of ultimate double bond relative to terminal methyl group.
[b] Sample slightly oxidized.
[c] NM = not mentioned.
[d] Includes both isomers.
[e] Private communication from D. F. Kuemmel.

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