# Studies on the Isomers of Major Monoenoic Acids in Rapeseed and Partially Hydrogenated Rapeseed Oil[1]

H.B.S. CONACHER and B.D. PAGE, Research Laboratories,
Health Protection Branch, Department of National Health and Welfare,
Ottawa, Ontario K1A OL2, Canada

## ABSTRACT

An analytical procedure is presented for studies on the composition of hydrogenated oils containing $C_{20}$ and $C_{22}$ fatty acids. The method involves an initial separation of esters by preparative gas liquid chromatography into fractions of equal chain length. Each fraction is subsequently studied in detail by gas liquid chromatography, argentation thin layer chromatography, IR spectroscopy, and microozonolysis. Results are presented from a study of the isomers of major monoenoic acids in commercial samples of rapeseed and partially hydrogenated rapeseed oils.

## INTRODUCTION

In the past few years nutritional studies on rapeseed oil have revealed unusual pathological changes in the hearts of several animal species (1-7). Compared to other commonly used vegetable oils, rapeseed oil contains substantial amounts of $C_{20}$ and $C_{22}$ monoenoic acids, and it has been suggested that the pathological changes might be due to the presence of these long chain acids, particularly the $C_{22}$ monoenoic (4,5).

Recent nutritional studies in our laboratories have included not only rapeseed oil, but also partially hydrogenated rapeseed oil and some partially hydrogenated marine oils (6,8). Since few data were available on the detailed fatty acid composition of these oils, it was considered important to obtain this information.

Analysis of the fatty acid composition of most nonhydrogenated vegetable oils is readily performed and that of rapeseed oil is well documented (9). In addition comprehensive studies of the same rapeseed oil (of composition slightly different from that analyzed in the present study) have been reported by Kuemmel (10) and by Ackman (11).

Partially hydrogenated vegetable oils, however, are more difficult to analyze because of numerous positional and geometrical isomers, many of which co-elute on conventional gas liquid chromatographic (GLC) columns. Most analytical schemes devised to date for such oils consist of a preliminary separation of esters into fractions according to unsaturation using counter-current distribution (12), mercury-adduct formation (13), or liquid chromatography on partially vulcanized rubber (14). Fractions are then separated by some form of silver ion chromatography into geometric isomers which are examined by oxidation and capillary GLC.

However these procedures have been applied to partially hydrogenated vegetable oils containing predominantly $C_{18}$ unsaturates, and little information is available on their successful application to oils containing $C_{20}$ and $C_{22}$ unsaturates. Lambertsen et al. (15), however, recently devised a scheme for the determination of monoene isomers in a partially hydrogenated marine oil, using reversed phase column chromatography as the preliminary separation step. Holmer and Jorgensen (16) have examined hydrogenated herring oil by silver ion thin layer chromatography, and Ackman et al. (17) have analyzed a similar oil by capillary GLC.

[1]Part of a paper presented at the AOCS Meeting, Atlantic City, October 1971.

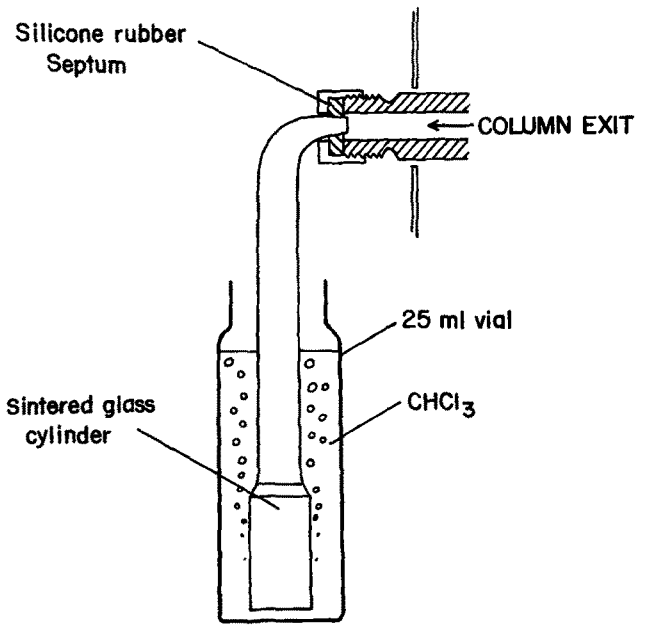


FIG. 1. Collection device for preparative gas liquid chromatography.

TABLE I

Composition of Esters of RSO and HRSO: Before and after Fractionation[a]

| | Area % composition | | | |
|---|---|---|---|---|
| | RSO | | HRSO | |
| Component | Before | After | Before | After |
| 16.0 | 4.9 | 4.1 | 3.0 | 3.0 |
| 16.1 | 0.2 | 0.1 | 0.1 | 0.1 |
| 18.0 | 1.8 | 1.6 | 7.2 | 7.2 |
| 18.1 | 23.7 | 23.0 | 36.2 | 36.4 |
| 18.2 | 21.6 | 21.2 | 10.1 | 9.3 |
| 18.3 | 7.0 | 6.4 | --- | --- |
| 20.0 | 0.6 | 0.8 | 2.5 | 2.1 |
| 20.1 | 11.0 | 11.8 | 10.9 | 10.4 |
| 20.2 | 0.5 | 0.2 | --- | --- |
| 22.0 | 0.4 | 0.3 | 4.0 | 4.3 |
| 22.1 | 28.4 | 30.5 | 26.0 | 27.1 |
| 22.2 | 0.1 | 0.1 | --- | --- |

[a]RSO = Rapeseed oil; HRSO = hydrogenated rapeseed oil.

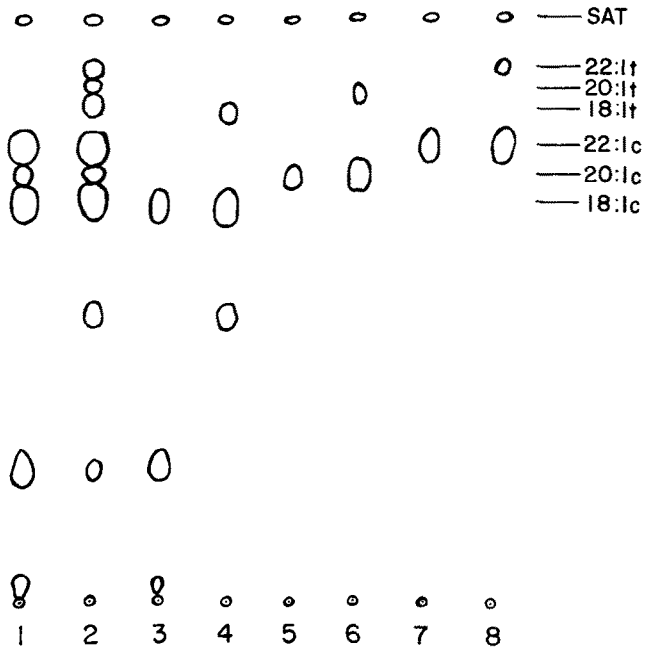


FIG. 2. Illustration of silver ion thin layer chromatographic separation of methyl esters of rapeseed oil (RSO), hydrogenated rapeseed oil (HRSO), and fractions. Solvent: chloroform (two developments) 1. RSO; 2. HRSO; 3. Fr18 (RSO); 4. Fr18 (HRSO); 5. Fr20 (RSO); 6. Fr20 (HRSO); 7. Fr22 (RSO), 8. Fr22 (HRSO). Components of $R_f$ less than 18:1c were not characterized.

The present report describes the procedures developed and the data obtained in an examination of the isomers of the major monoenes in a rapeseed and a partially hydrogenated rapeseed oil. The technique involves an initial separation of the esters by preparative GLC into fractions according to chain length, and subsequent analysis of each fraction by IR spectroscopy (18, 19), silver ion thin layer chromatography (TLC) and microozonolysis (20,21).

## MATERIALS AND METHODS

Commercial samples of rapeseed oil (RSO) and partially hydrogenated rapeseed oil (HRSO) were obtained from Canada Packers Ltd. The latter oil was similar to that used in shortenings and margarines in Canada and had an iodine value of 78. Methyl esters were prepared by transesterification with 1% sulfuric acid in anhydrous benzene-methanol 1:3.

### Gas Liquid Chromatography

Direct analyses of methyl esters were carried out on a 6 ft x 1/8 in. stainless steel column packed with 20% DEGS (diethylene glycol succinate) and (in part) on a 5 ft x 1/8 in. stainless steel column packed with 3% SE30.

Aldehyde-esters from ozonide decompositions were analyzed on the same columns, temperature programed from 70-190 C (DEGS) and from 70-250 C (SE30).

TABLE II

Isomers of Major Monoenoic Acids in Rapeseed Oil

| Fraction | Monoene, %[a] | Composition of aldehyde-esters, mole % | | | | |
|---|---|---|---|---|---|---|
| | | 9 | 10 | 11 | 12 | 13 |
| 18 | 23.7 | 94 | --- | 6 | --- | --- |
| 20 | 11.0 | --- | --- | 93 | --- | 7 |
| 22 | 28.4 | --- | --- | --- | --- | 100 |

[a]Per cent of total oil.

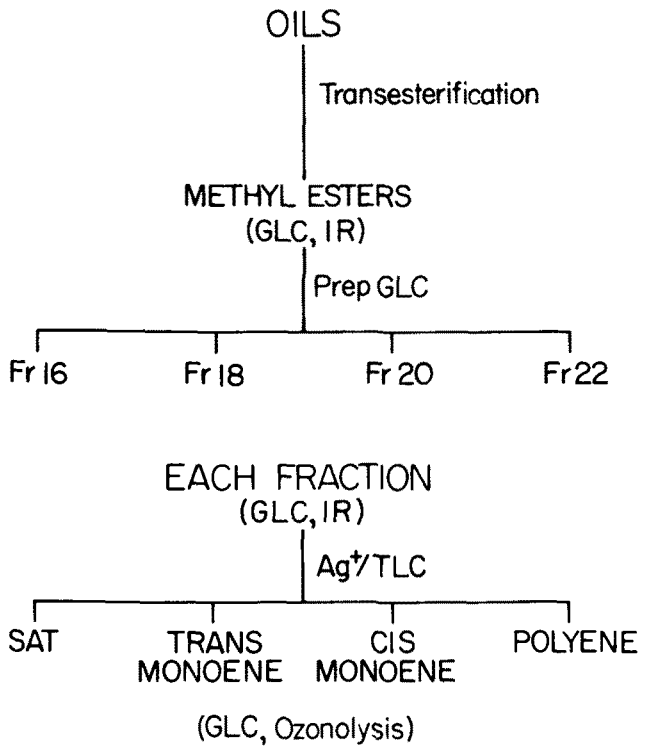


FIG. 3. Scheme for separation and analysis.

### Preparative Gas Liquid Chromatography (prep GLC)

Methyl esters were fractionated at 200 C on an Aerograph A-90 instrument equipped with a flame ionization detector and a 6 ft x 1/4 in. glass column packed with 10% SE30 on 60-80 mesh Chromosorb W. Fractions of equal chain length were collected in chloroform using glass tubes (90 mm length) fitted at one end with sintered glass cylinders (12 mm diam x 25 mm, coarse porosity) (Fig. 1). Variations in porosity which resulted in varying split ratios were normalized by varying chloroform levels in relevant collection vials to give a split-ratio of 3:1 in favor of the collector. Injections of 8-10 mg gave suitable separations.

To ensure that no positional or geometrical isomerization was occurring during fractionation, samples of high purity oleate, elaidate and linoleate were examined before and after collection by GLC, TLC, IR and ozonolysis. No changes were observed. Also GLC analysis of the recombined fractions from both RSO and HRSO showed that no selective loss within fractions was occurring. These general fatty acid compositions are summarized in Table I.

### Silver Ion Thin Layer Chromatography ($Ag^+$/TLC)

Fractions were separated into *cis* and *trans* isomers on 500 $\mu$ layers of Silica Gel G containing 12% silver nitrate. The addition of methyl pentadecanoate to each band prior to elution (ether for monoenes; ether saturated with water for polyenes) permitted quantitation by GLC.

### Ozonolysis

Double bond location in monoenes was determined by ozonization in a Supelco Micro-ozonizer. Samples (100 $\mu$g) in carbon disulfide (100 $\mu$liters) were ozonized at -70 C for 30 sec with an oxygen flow rate of 10 ml/min. Ozonides were decomposed in situ with tetracyanoethylene (21) and relative amounts of isomers calculated from GLC analysis of the aldehyde-ester fragments.

### IR Spectroscopy

*Trans* contents were determined by comparison of IR absorptions at 965 $cm^{-1}$ *(trans)* and at 1730 $cm^{-1}$ (carbonyl) as described by Allen (18), and also by the AOCS

TABLE III

Silver Ion Thin Layer Chromatographic Separation of Fractions From Hydrogenated Rapeseed Oil

| | Area % composition | | | | |
|---|---|---|---|---|---|
| | | Monoene | | | |
| Fraction[b] | Sat | *trans* | *cis* | Diene | IR[a] |
| 18 (GLC) | 13.1 | | 71.0 | 15.9 | |
| (TLC) | 13.1 | 22.6 | 47.6 | 16.7 | 32.2 |
| 20 (GLC) | 18.0 | | 82.0 | --- | |
| (TLC) | 21.2 | 19.0 | 60.8 | --- | 17.0 |
| 22 (GLC) | 13.7 | | 86.3 | --- | |
| (TLC) | 12.4 | 17.4 | 70.2 | --- | 16.0 |
| IR (original | | | | 24.4 % *trans* | |
| IR (combined) | 3.1[c] (0.0) + 53.5 (32.2) + 13.4 (17.0) + 30.0 (16.0) = | | | 24.3 % *trans* | |

[a]Per cent *trans* content calculated from IR.

[b]GLC = Gas liquid chromatography; TLC = thin layer chromatography.

[c]Figures preceding parentheses represent per cent of each fraction in oil. 3.1 represents $C_{16}$ acids of negligible *trans* content and not listed in table.

standard procedure (19).

## RESULTS AND DISCUSSION

The general fatty acid compositions of RSO and HRSO are presented in Table I. The major apparent changes are a reduction in polyene content (29% to 10%), an increase in saturates (8% to 17%), and an increase in the $C_{18}$ monoenoic acids (24% to 36%). There is little change in both the $C_{20}$ and $C_{22}$ monoene contents.

In the initial stages of this work attempts were made to separate the esters of RSO and HRSO by $Ag^+$/TLC using a variety of solvent systems. A typical separation of monoenes from RSO and HRSO is illustrated in Figure 2. Because of the separation due to chain length being superimposed upon that due to geometrical configuration, fractions of sufficient purity for ozonolysis could not be reproducibly obtained.

The scheme of separation and analysis finally adopted for these oils is illustrated in Figure 3. These procedures provide data for calculating the percentage of all *cis* and *trans* positional isomers in the oil samples.

Although prep GLC has been applied previously (22,23) in the isolation of similar fractions from lipids, it was essential to ensure that no isomerization was occurring during fractionation. The steps taken to demonstrate this have been described in the experimental section.

### Rapeseed Oil

Application of the analytical scheme to rapeseed oil gave the monoene isomers shown in Table II. These isomers are qualitatively similar to those observed previously (10,11), except that here the $C_{22}$ monoene comprised only one isomer, viz. erucic acid.

### Partially Hydrogenated Rapeseed Oil

Table III shows the data obtained from $Ag^+$/TLC separation of the major fractions from HRSO, together with the *trans* content of each fraction as determined by IR spectroscopy.

The total *trans* content calculated from that of each fraction (24.3%) shows excellent agreement with that of the original methyl esters (24.4%). Slight discrepancies in agreement between the *trans* contents, determined by $Ag^+$/TLC and by IR, for both Fractions 20 and 22, are probably due to calibration of the IR technique with elaidate. For Fraction 18, comparison is invalidated due to *trans* contribution from the diene isomers in the IR measurements.

The monoene isomers present in each major fraction from HRSO are summarized in Table IV. From these data it can be observed that *cis* monoenes in all fractions reflect favorably the composition, with respect to double bond position, of corresponding fractions in RSO (Table II), with the addition of minor amounts of newly formed *cis* double bonds. The 18 *cis* monoene (HRSO) also contains 11% of the $\Delta 12$ isomer, presumably derived from reduction of the 9 double bond in linoleate. However the $\Delta 15$ isomer, which could derive from linolenate, was not observed.

In the *trans* monoenes, double bonds are more widely scattered as has been previously demonstrated in other partially hydrogenated oils (12-14). With the 20 and 22 *trans* fractions, both derived essentially from monoenes, two major isomerizations have occurred: a conversion from *cis* to *trans*, with double bond retaining its original position; and a migration, equally in both directions, to give *trans*

TABLE IV

Isomers of Major Monoenoic Acids in Hydrogenated Rapeseed Oil

| | Composition of aldehyde-esters, mole % | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fraction | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| 18 (36.2%)[a] | | | | | | | | | |
| *trans* (32%)[b] | 1 | 8 | 38 | 18 | 14 | 12 | 5 | 2 | 2 |
| *cis* (68%) | --- | 2 | 79 | 2 | 6 | 11 | --- | --- | --- |
| 20 (10.9%) | | | | | | | | | |
| *trans* (24%) | --- | --- | 1 | 12 | 59 | 15 | 8 | 4 | 1 |
| *cis* (76%) | --- | --- | --- | 4 | 86 | 2 | 8 | --- | --- |
| 22 (26.0%) | | | | | | | | | |
| *trans* (20%) | --- | --- | --- | --- | 1 | 15 | 66 | 16 | 2 |
| *cis* (80%) | --- | --- | --- | --- | --- | 3 | 95 | 2 | --- |

[a]Per cent in parentheses after fractions refer to monoene content of total oil.

[b]Per cent in parentheses after *trans* and *cis* refer to per cent within monoene fractions.

bonds on either side of the original bond location. This is in agreement with previous observations (24,25). In the case of the 18 *trans* monoenes the situation is more complicated because these derive from both original oleate and from polyenes. However a similar pattern is apparent.

In addition there appears to be slightly more *trans* formation in the 20 fraction (24%) than in the 22 fraction (20%). This may be due to the larger molecular weight and higher viscosity of the latter fraction. Similar observations have been made on the isomerization of glycerides compared to their ester counterparts, the former isomerizing about 5% more sluggishly (26).

From the data presented in Tables II and IV, the percentage of any monoenoic isomer in the oils can be calculated. An appropriate illustration would be erucic acid. From Table II the percentage of erucic acid in RSO is 28.4%. From Table IV the percentage in HRSO is 26.0% x 80% x 95% = 19.8%. Similarly the major 20 monoene in RSO is 11% x 93% = 10.2%, whereas in HRSO the percentage is 10.9% x 76% x 86% = 7.1%.

Thus the amounts of both original *cis* monoenes are reduced, under the commercial hydrogenation conditions, by about 30%.

Similar calculations provide for a complete analysis of monoenes in terms of double bond position and geometric configuration.

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