Some Minor Fatty Acids of Rapeseed Oils

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ABSTRACT

A number of minor unsaturated fatty acids of rapeseed oil (from *Brassica napus* or *campestris*) have been isolated by combinations of distillation, preparative gas liquid chromatography and silver nitrate thin layer chromatography, and were further identified by oxidative fission in BF_3-MeOH. Among the shorter chain, all-*cis* polyunsaturated fatty acids described are 16:3ω3, 16:2ω6 and 14:2ω6. A ubiquitous minor component in *unprocessed* oils was found to be *cis-9, cis-12, trans-15*-octadecatrienoic acid, with lesser proportions of the *trans-9, cis-12, cis-15* isomer. Among others identified were *cis*-14:1ω9 and 15:1ω10, the latter accompanied by half as much *trans*-15:1ω10. Particular attention was paid to the proportions of the minor monoethylenic fatty acids of the ω7 series relative to the longer chain major ω9 monoethylenic fatty acids which have been reduced by plant breeding.

INTRODUCTION

Oils from the seeds of the rapeseed plant (Canadian commercial species are normally *Brassica napus* or *Brassica campestris*) have conventionally been characterized by high proportions (25-45%) of the erucic (*cis*-13-docosenoic or 22:1ω9[1]) acid (1-3). The other major fatty acids are *cis*-9-octadecenoic, *cis*-11-eicosenoic acid, *cis, cis*-9,12-octadecadienoic acid and *cis, cis, cis*-9,12,15-octadecatrienoic acids. The saturated acids 16:0, 18:0, 20:0 and 22:0 are relatively much less important, and together these fatty acids account for about 7% of total fatty acids. In experimental animals, especially male rats, the feeding of rapeseed oils is reported to give rise to cardiomyopathy (4-7). This was initially attributed to erucic acid *per se*, but recently other oils and newer varieties of rapeseed oils low in erucic acid have in some laboratories shown similar if milder cardiomyopathies in the laboratory rat (5,6,8,9). As some controversy surrounds these results, we have carried out a detailed study of the minor fatty acids of rapeseed oil. This report describes the specific identification and quantitation of 22:1ω7 (*cis*-15-docosenoic), 20:1ω7 (*cis*-13-eicosenoic), and 18:1ω7 (*cis*-11-octadecenoic or *cis*-vaccenic) acids; 16:3ω3 (*cis, cis, cis*-7,10,13-hexadecatrienoic acid); 16:2ω6 and 14:2ω6 (*cis, cis*-7,10-hexadecadienoic and *cis, cis*-5,8-tetradecadienoic acids), 14:1ω9 (*cis*-5-tetradecenoic acid) and both *cis*- and *trans*-15:1ω10 (*cis*- and *trans*-5-pentadecenoic acids). Several other minor acids have been concentrated and tentatively identified.

EXPERIMENTAL PROCEDURES

Oils

Three crude rapeseed oils were selected for study. One represented the conventional high erucic oils formerly grown in quantity in western Canada (probably from Target, a *B. napus* variety). Two representatives of the low erucic varieties were Span, a *B. campestris* variety and Tower, a *B. napus* variety, both of which have been used extensively in animal trials. All oils had been commercially extracted with hexane from crushed seed and given centrifugal and washing treatments to eliminate water and a certain proportion of gums, but had not been further processed in any way. Selected seed samples were simply crushed and the oil extracted with boiling hexane.

Thin Layer and Gas Liquid Chromatography (TLC and GLC) and Distillation

Prekotes (Adsorbosil-5; Applied Science Laboratories) dipped in a solution of silver nitrate (10%) in acetonitrile, then dried, were employed for the thin layer chromatographic separations of the methyl esters by degree of unsaturation. Development was in benzene/hexane (2:1) followed by a 2,7-dichlorofluorescein spray and immediate visualization under UV light. Bands were scraped off and the silica gel extracted several times with hexane and $CHCl_3$ to maximize recovery.

Preparative GLC was executed on a Varian Autoprep with a stainless steeel column 2 m in length and 4 mm I.D. packed with Chromosorb-W coated with 5% SE-30. Analytical GLC, including ozonolysis products (10), was executed on stainless steel, wall-coated, open tubular columns 47 m in length and 0.25 mm I.D., coated with SILAR-5CP or Apiezon-L (AP-L) and operated in a Perkin-Elmer 900 series apparatus with flame ionization detector.

The methyl esters of crude Tower low erucic acid rapeseed oil were distilled under high vacuum through a Stedman column (11) to obtain a fraction consisting mostly of esters more volatile than the C_{18} chain length.

Chemical Preparations

Oils were saponified and nonsaponifiables were removed by AOCS procedure Ca-6a-40. The recovered fatty acids were converted to methyl esters by refluxing for six hr with 10 volumes of 5% H_2SO_4 in absolute MeOH. The esters were recovered and washed free of acids with sodium bicarbonate solution.

Ozonolysis basically followed procedures published for monoethylenic fatty acids (10) but was slightly modified for polyunsaturated fatty acids (12).

RESULTS

Minor Polyunsaturated Fatty Acids

$AgNO_3$-TLC of a distillate cut of methyl esters of Tower oil primarily of C_{12}, C_{14}, and C_{16} material with some C_{18} (Fig. 1), developed with the solvent system benzene/hexane (2:1), gave clean separation of triene, diene, monoene and saturated ester bands.

The triene band when examined by analytical GLC contained two major components with a molar ratio of 1:1 if calculated as 16:3 and 18:3. Ozonolysis gave primarily two major diacidic products, DMC_7 (dimethyl pimelate; dimethyl heptanedioate) and DMC_9 (dimethyl azelate; dimethyl nonanedioate) in a mole ratio of 1:1.2. The trienoic isomers present were thus identified as most probably 16:3ω3 and 18:3ω3 and on the basis of single peaks and of GLC retention data (Table I) were considered to be all *cis* and unaccompanied by other isomers. No attempt was made to isolate 20:3ω3 and 22:2ω3 compo-

[1]Shorthand notation for chain length, number of ethylenic bonds and position of bond closest to terminal methyl group. In polyethylenic acids, systems are methylene interrupted, and if not otherwise stipulated, bonds are *cis* in geometry.

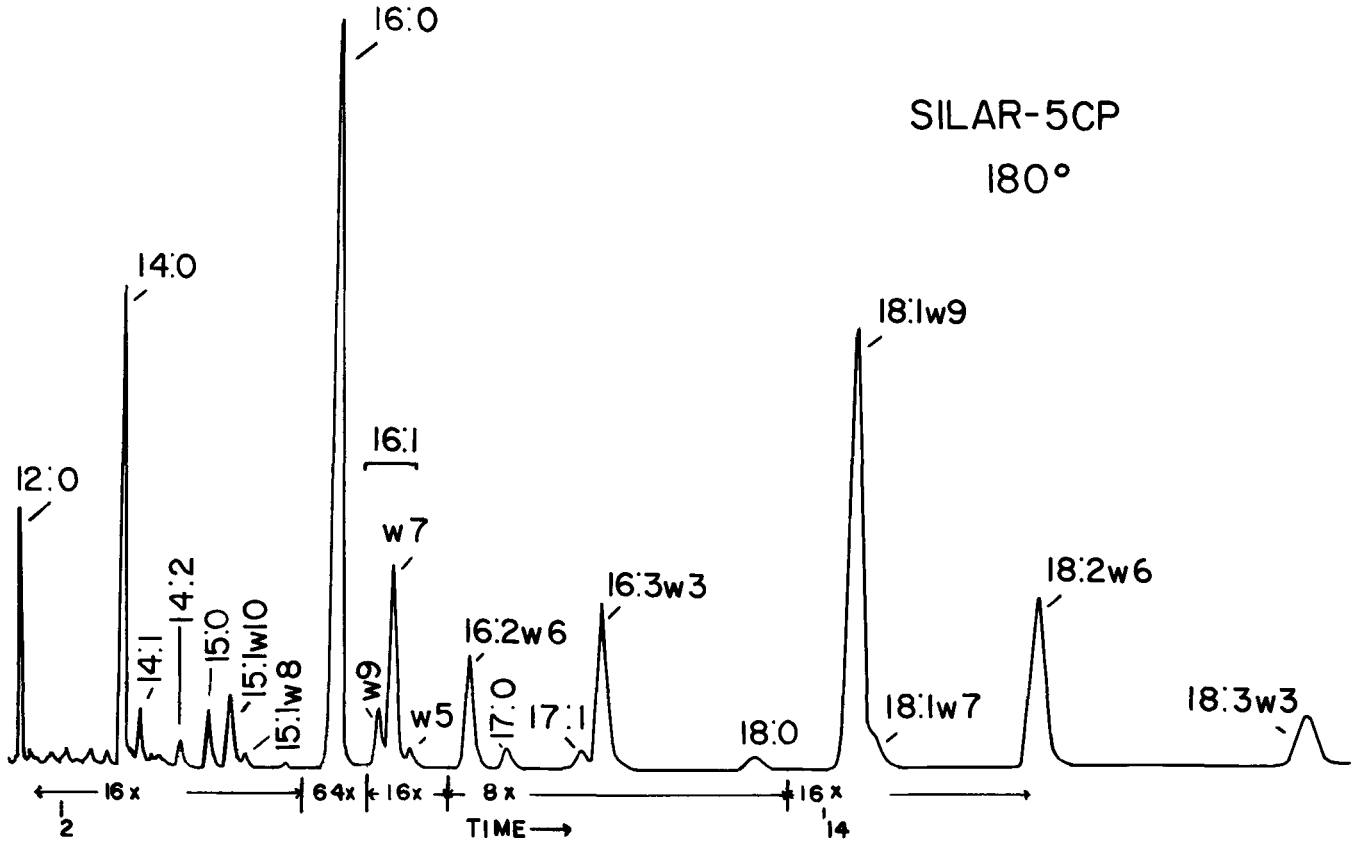

FIG. 1. GLC analysis on SILAR-5CP of distillate fraction of methyl esters of fatty acids from Tower rapeseed oil. Minor peaks are listed in Table I and are discussed in the text.

TABLE I

Retention data on AP-L at 185 C and on SILAR-5CP at 180 C for some minor fatty acids of Tower rapeseed oil, and proportions in whole oil

Observed ECL		Identification			
AP-L	SILAR-5CP	Monoene	Diene	Triene	w/w%
13.67	14.22	14:1ω9	---	---	---
13.69	14.41	14:1ω7	---	---	0.007
13.79	--	14:1ω5	---	---	---
13.50	14.69[a]	---	14:2ω6	---	0.004
15.00	15.00	---	---	---	0.02
14.64	15.21	(*cis*)15:1ω10	---	---	0.02
14.78	15.21	(*trans*)15:1ω10	---	---	0.01
14.65	15.35	15:1ω8	---	14:3ω3	---
15.64	16.31	16:1ω9	---	---	---
15.70	16.40	16:1ω7	---	---	0.29
15.78	16.51	16:1ω5	---	---	---
15.50	16.81	---	16:2ω6	---	0.07
17.00	17.00[b]	---	---	---	0.05
16.65	17.31	17:1ω8	---	---	0.06
15.48	17.41	---	---	16:3ω3	0.13
17.62	18.28	18:1ω9	---	---	---
17.68	18.34	18:1ω7	---	---	---
17.45	18.78	---	18:2ω6	---	---
17.42	19.38	---	---	18:3ω3	---
--	~19.28	19:1	--	--	0.02

[a]Includes anteiso 15:0.
[b]Includes 16:2ω4.

nents indicated by open tubular GLC (3) and other studies (13,14).

The diene band on $AgNO_3$-TLC showed two densities, and the bottom half was extracted specifically to reduce the proportion of the major component 18:2. Analytical GLC of this enriched fraction gave weight percentages corresponding to 14:2, 16:2, and 18:2 of 18%, 66% and 16%, and on hydrogenation the esters of the saturated acids recovered were 14:0, 16:0 and 18:0, respectively, 19%, 62% and 19% by weight. The 16:2 component appeared to have about 5% of a trailing shoulder which disappeared on hydrogenation.

The longer chain diacidic products of ozonolysis of the enriched diene band portion were DMC_7 accompanied by DMC_9 in a proportion of about 4:1. Clearly the principal diene was 16:2ω6, and not unexpectedly the 18:2 component was 18:2ω6. The 14:2 component (Fig. 1) was isolated by preparative GLC. The amount available was very

TABLE II

Fractional Chain Lengths, Diene Adjustments, and Calculation of Equivalent Chain Lengths for 14:2ω6 on AP-L at 185 C and SILAR-5CP at 180 C

	AP-L	SILAR-5CP
FCL for Δ5[a]	0.64	0.21
FCL for ω6[b]	0.73	0.42
Diene adjustment[b]	0.13	0.04
Calculated ECL	13.50[c]	14.67[d]
Experimental ECL[a]	13.50	14.69

[a]Based on 15:1 data from Table I for reasons of accuracy.
[b]Values taken from laboratory publications (15-17).
[c]14.00 - (0.36 + 0.27) + 0.13 (15).
[d]14.00 + 0.21 + 0.42 + 0.04 (16).

small but yielded only 14:0 on hydrogenation, and DMC_5 on ozonolysis with extraction of diacid esters from the ozonolysis-oxidation medium by $CHCl_3$. Table II gives calculations (15-17) for ECL values supporting the identification of 14:2ω6. The early elution on SILAR-5CP is due to the presence of the Δ5 bond (compare monoene retention data, Table I). The minor 16:2 shoulder could be 16:2ω4, a fatty acid known to be found in marine algae (18) and which would contribute DMC_9 to the reaction products, or some similar isomer (note that enrichment within 16:2 isomers on $AgNO_3$-TLC is possible).

The methyl esters from the crude Tower oil methyl ester distillates rich in 18:3 showed shoulder components on each side of 18:3ω3 previously reported (19) in refined edible oils as 18:3 isomers "A" and "C". These were isolated (see below) by $AgNO_3$-TLC as described earlier (19), and on partial hydrazine reduction the products gave the same geometrical and positional isomer mixture GLC pattern on the liquid phase SILAR-5CP as that published earlier. This strongly suggests that A is methyl *cis-9, cis-12,-trans*-15-octadecatrienoate, and C is methyl *trans-9, cis-12, cis*-15 octadecatrienoate. A + C were < 1% of the total 18:3 in early distillate cuts but increased steadily in later distillate cuts to as much as 20% of total 18:3ω3.

Since isomers A and C were present in the earliest distillate esters containing 18:3ω3, a number of oils were extracted directly from crushed raw rapeseed by refluxing in hexane. These were from *B. campestris* varieties, Span and "yellow seed coat," and *B. napus* varieties, Midas and Tower. The oils were converted to methyl esters by the Christopherson and Glass alkali-catalyzed transesterification (20), or by heating in 7% BF_3-MeOH:hexane (1:1) for 1 hr. The esters were streaked on $AgNO_3$-TLC plates, and the plate area *between* the obvious triene and diene bands was recovered with hexane and $CHCl_3$. The peak for A was in all cases present in an excess of between 5:1 and 10:1 relative to C (Fig. 2). Since esterification of pure 18:3ω3 (Hormel Institute) by the BF_3-MeOH procedure did not produce corresponding isomerization to A and C, it must be concluded that isomerization of any of the *cis*-ethylenic bonds in 18:3ω3 can take place in the seed, but that the more exposed or more labile Δ15 (or ω3) bond is particularly affected. In analyses of methyl esters prepared from whole oil on SILAR-5CP, there is coincidence of A and 19:1 (see below), but on SILAR-7CP isomer A is free of this interference (not shown) and particularly well separated from all-*cis* 18:3.

Minor Monoethylenic Fatty Acids

Analysis of the distillate fraction (Fig. 1) on AP-L (Fig. 3) gave confirmation of the presence of the three 16:1 isomers and of 16:2ω6 and 16:3ω3. Unexpectedly, the region corresponding of 15:1 showed three components instead of the one major and one minor component of the

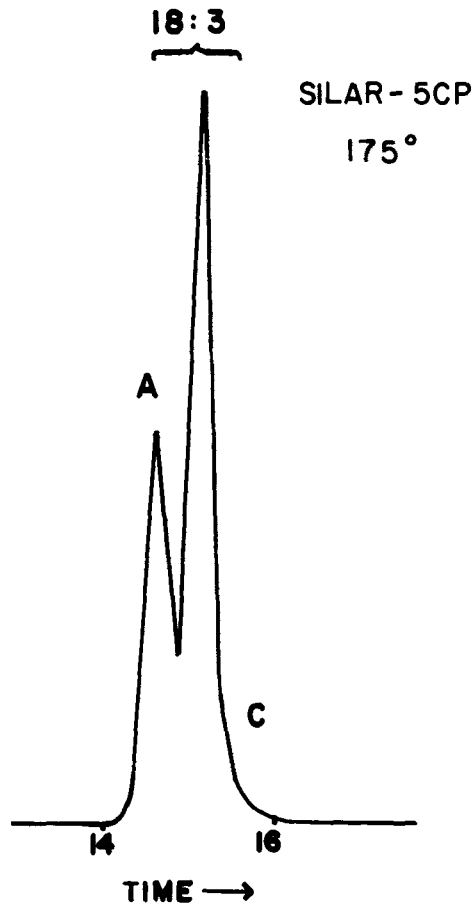

FIG. 2. Part of GLC analysis on SILAR-5CP of methyl esters of fatty acids from $AgNO_3$-TLC area *between* diene and triene bands (including small proportions of the two observed bands). Sample: Tower low erucic acid rapeseed, laboratory extract. Isomer C is the shoulder on the trailing edge of the major peak.

SILAR-5CP analysis (Fig. 1). The smallest of these on AP-L was later shown to be *anteiso*-15:0. Relating the quantitation of the three unsaturated peaks to the adjacent 15:0 showed that the minor peak at ECL 15.35 on SILAR-5CP, tentatively identified as 15:1ω8, was included in the largest peak at ECL 14.64 on AP-L. However, the supposed 15:1ω10 eluting earlier on SILAR-5CP at ECL 15.21 had definitely split into two components in a ratio of 2:1 with respective ECL values on AP-L of 14.64 and 14.78. The fraction containing 15:1 was further enriched by preparative GLC and subjected to $AgNO_3$-TLC. A separate monoene band lying between the main monoene band and the saturated ester band corresponded to the lesser AP-L peak at ECL 14.78. Ozonolysis gave only DMC_5 (dimethyl glutarate; dimethyl pentanedioate) and MMC_{10} (methyl caprate; methyl decanoate) as products. The sole fatty acid present in this band was therefore 15:1ω10 (5-pentadecenoic acid) and by analogy to the behavior of 16:1ω10 on Apiezon-L (21), and from its position in $AgNO_3$-TLC, had to be the *trans* isomer. However, no infrared data could be obtained due to lack of sample.

The principal $AgNO_3$-TLC monoene band from this enriched Tower fraction when analyzed on SILAR-5CP contained the other (and larger) 15:1 component, plus the principal 14:1 component of ECL 13.67 on AP-L and two minor 14:1 components (Fig. 4) as shown by qualitative and quantitative analytical GLC before and after hydrogenation. Ozonolysis gave DMC_5, MMC_{10} and MMC_9 (methyl pelargonate, methyl nonanoate) in proportions confirming the principal components as 14:1ω9 and

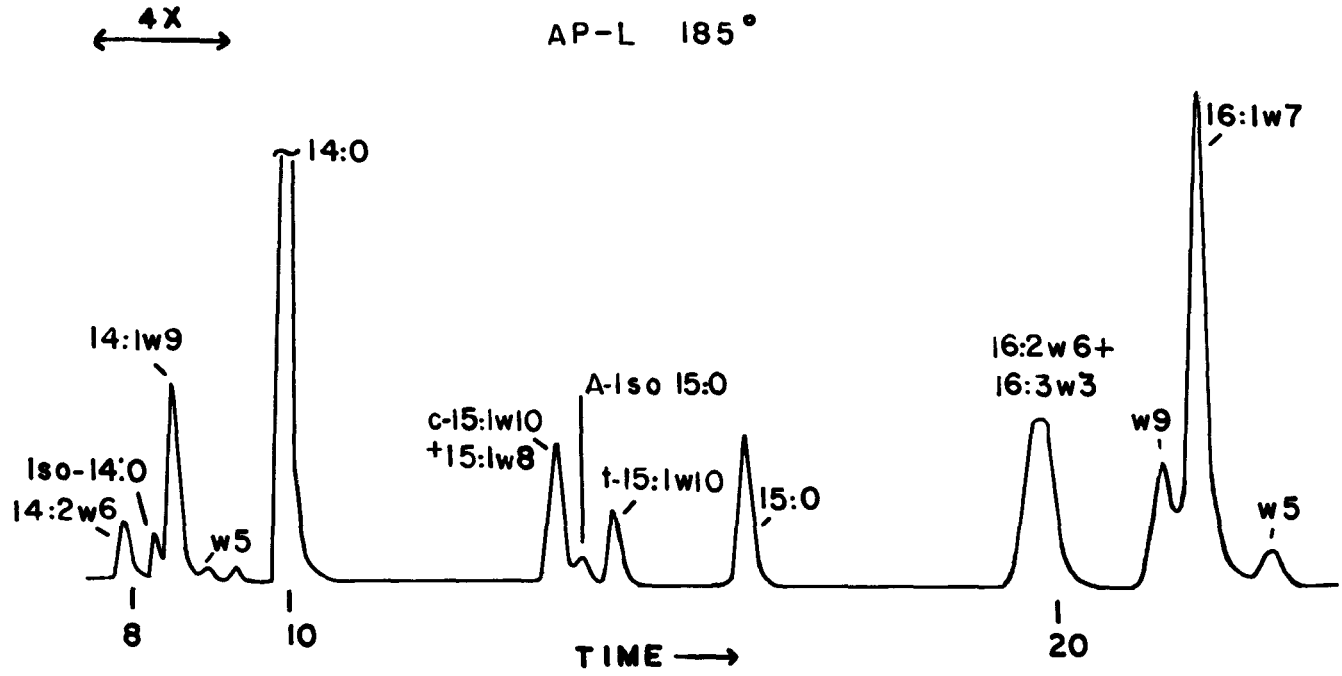

FIG. 3. Part of GLC analysis on Apiezon-L of methyl ester fraction (from Tower rapeseed oil) analysed for Fig. 1. Note partial separation of 16:3ω3 and 16:2ω6.

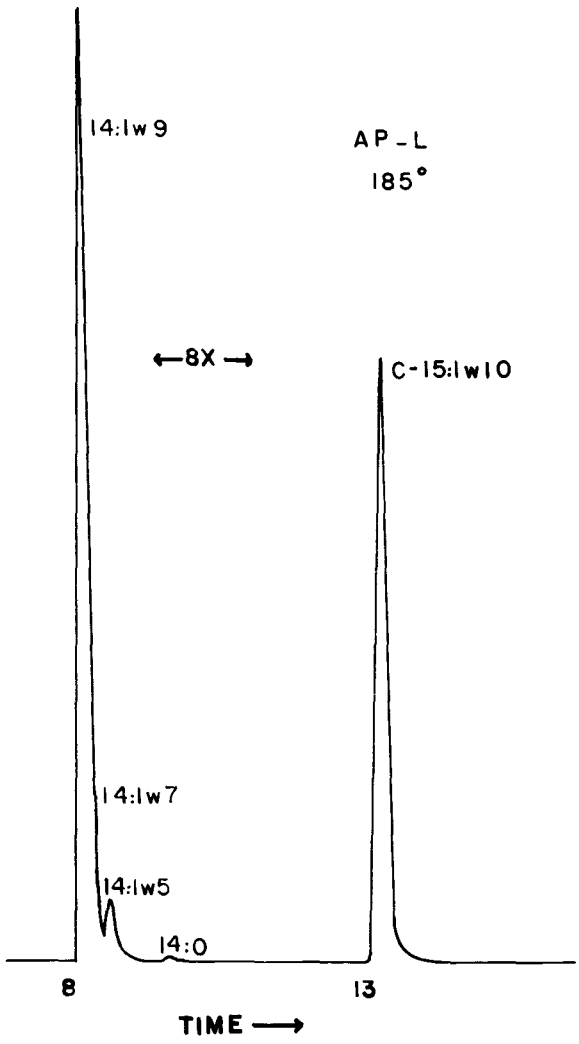

FIG. 4. Part of GLC analysis on Apiezon-L of *cis*-monoene band from $AgNO_3$-TLC of preparative GLC cut of methyl esters of fatty acids of Tower rapeseed oil.

15:1ω10. The $AgNO_3$-TLC behavior showed these two components to be *cis* in structure. A small DMC_7 ozonolysis product confirmed that the minor 14:1 isomer in the same band was *cis*-14:1ω7. This isomer was well separated from 14:1ω9 (11) on SILAR-5CP but was barely visible as a shoulder on the back of the 14:1ω9 peak on AP-L (Fig. 4). The 14:1ω5 isomer was tentatively identified from GLC data (Table I).

The single 17:1 isomer present in whole oil esters and concentrates (Fig. 1) was identified from GLC retention behavior as the ω8 isomer. The presence of one or more 19:1 isomers was confirmed after $AgNO_3$-TLC concentration, but the sample was very limited and no discrimination between the ω8 and ω10 isomers was possible.

Distribution of Even Chain Length Monoethylenic Isomers

The separation of Span oil methyl esters by $AgNO_3$-TLC with benzene/hexane (2:1) as solvent gave a superficially distinct band for 22:1. However, analysis of the recovered band showed the actual mole % composition by chain lengths to be 18:1ω9 + 7, 1%; 20:1ω9 + 7, 2.8%; 22:1ω9 + 7, 93.6% and 24:1ω9, 2.6%. This sample was subjected to ozonolysis, and after correction of DMC_{15} (dimethyl pentadecanedioate) for that derived from 24:1ω9, it was calculated that the 22:1 isomer proportions were 98.2%, 22:1ω9 and 1.8%, 22:1ω7.

The C_{22} methyl esters from the high erucic acid rapeseed oil were collected from preparative GLC. The product consisted of 22:1 and 22:0 in proportions of 98.5:1.5 with only traces of 18:1, 18:2 and 20:1, and no detectable 24:1. Ozonolysis gave DMC_{15}, DMC_{13} (dimethyl ester of brassylic acid, dimethyl tridecanedioate) and unaltered 22:0. Traces of DMC_{11}, DMC_{12} and DMC_{14} were also observed but did not exceed expected levels for artifacts and contaminants. The whole procedure was repeated once on this sample, and the same procedure was then applied three times to the methyl esters of Tower low erucic acid rapeseed oil. The average isomer proportions of 22:1ω9 and 22:1ω7 were 99.1:0.9 for high erucic acid rapeseed oil and 97.7:2.3 for Tower low erucic acid rapeseed oil. The procedure was then applied in duplicate to the C_{20} chain

TABLE III

Percentages for Total Even Chain Length Monoethylenic and Saturated Fatty Acids, with Distribution of Monoethylenic Fatty Acid Isomers in Each Chain Length[a], for High and Low Erucic Acid Rapeseed Oils

	High erucic oil[b]		Low erucic oil[c]	
Fatty acid	% Isomers	% of Total Acids	% Isomers	% of Total Acids
24:0	—	0.3	—	0.2
24:1ω9	90	1.0	70	0.2
24:1ω7	10		30	
22:0	—	0.4	—	0.2
22:1ω9	99.1	23	97.7	0.1
22:1ω7	0.9		2.3	
20:0	—	0.5	—	0.5
20:1ω9	95.6	11	97.6	1.2
20:1ω7	4.4		2.4	
18:0	—	1.2	—	1.4
18:1ω9	95.1	35	95.6	64
18:1ω7	4.9		4.4	
16:0	—	2.6	—	3.9
16:1ω9	13	0.2	22	0.3
16:1ω7	78		70	
16:1ω5	9		8	

[a]24:1 and 16:1 isomers estimated from peak height ratios, others from ozonolysis.
[b]Variety Target.
[c]Variety Tower.

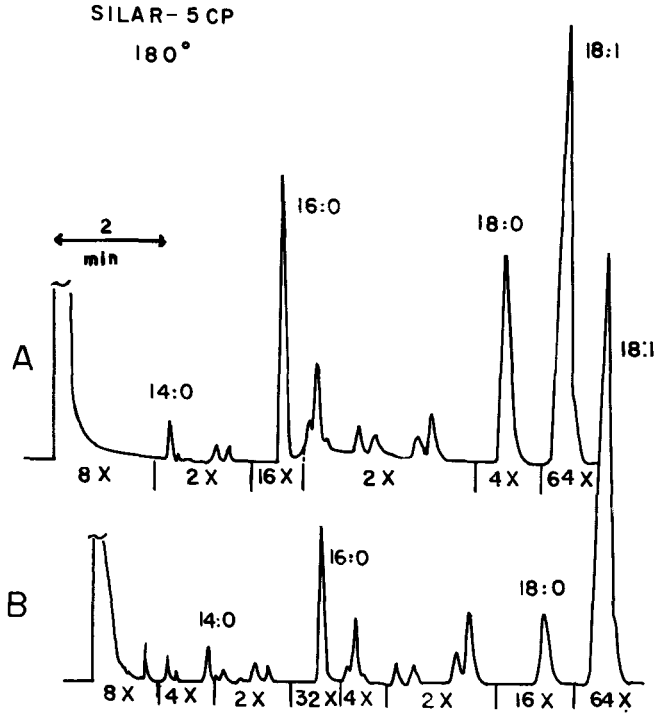

FIG. 5. Parts of GLC analyses on SILAR-5CP of methyl esters of fatty acids of shorter chain lengths from high erucic (B, variety Target) and low erucic (A, variety Tower) rapeseed oils. The similarities (note attenuation changes) are apparent.

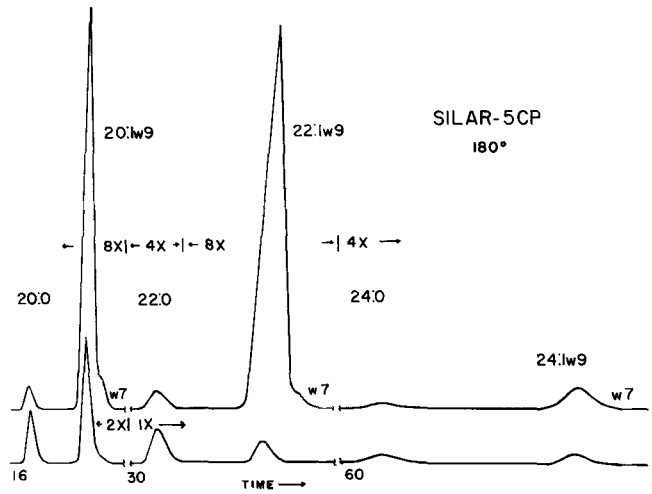

FIG. 6. Continuations of chart records shown in Fig. 5, reversed, showing differences in the methyl esters of fatty acids of longer chain lengths. Above, high erucic (variety Target); below, low erucic (variety Tower); liquid phase, SILAR-5CP.

length esters and the C_{18} chain length esters of these two oils. For the C_{18} chain length, an additional step of $AgNO_3$-TLC was applied after preparative GLC. Great care was taken to recover all 18:1 esters from the TLC plate. This definitive isomer distribution data is presented in Table III along with 16:1 isomer proportions taken from GLC analyses.

Minor Saturated Acids

The saturated acid ester band from $AgNO_3$-TLC of the Tower distillate confirmed the presence of a trace of 13:0 and definitive amounts of 15:0 and 17:0 (Fig. 1, Table I).

In addition, two small components were detected with ECL values of 13.67 and 14.70 on Ap-L and of 13.58 and 14.69 on SILAR-5CP. The literature retention data (22) clearly indicates these to be respectively *iso*-14:0 (Fig. 3) and *anteiso*-15:0 (Fig. 3) as marked.

DISCUSSION

From a nutrition point of view, the marked diminution of total 22:1 from 30-50% to as little as 0.1% of total fatty acids in rapeseed oil (2) is a major triumph of plant breeding (23), and for practical purposes has created a new edible oil (2). Low erucic acid (22:1<5%) rapeseed oil is being given a standard of identity under the Codex Alimentarius.

In Canada the term "Canola" has been adopted to specifically designate seed, or products from seed, of varieties very low in both erucic acid and glucosinolates.

In the course of this development, the shorter chain, minor fatty acids of rapeseed oil (Table I) have changed very little. Open tubular, gas liquid chromatographic charts

can be matched in almost all details for chain lengths shorter than C_{18} (Fig. 5). Despite prior publication (3) of open tubular gas chromatographic and other analytical data showing that 18:1ω9, 20:1ω9 and 22:1ω9 were accompanied by 18:1ω7, 20:1ω7 and 22:1ω7 (compare Fig. 6), at least one report states that there is no 22:1ω7 in rapeseed oil (14). The proportions of 20:1 and 22:1 are now so reduced (Fig. 6) that it is difficult to study isomer proportions by gas liquid chromatography without overloading the column in respect to C_{18} components. Table III gives figures for the variety Tower. The variety Span 22:1 data (see results) show an intermediate ω9:ω7 isomer proportion of 98.2:1.8 in a total 22:1 of 3%.

An interesting feature of the isomer distribution pattern of Table III is that total ω7 isomers are slightly higher in the low erucic acid oils (3.1%) than in the high erucic acid oils (2.6%). The gene responsible for 4-carbon addition to 18:1ω9 to give 22:1ω9 probably also produced 20:1ω7 from 16:1ω7, and with the elimination of this gene the proportion of 20:1ω7 to 20:1ω9 is notably reduced in the low erucic acid oils. The percentages of 24:0 and 24:1 (Fig. 6) in total acids of all really low erucic acid rapeseed oils examined (2) fall into a group of about 0.1% of total fatty acids (except for Span, with 0.1 and 0.3%, respectively).

Publication of claims that the major 22:1 component of rapeseed oil is *trans*-22:1ω9 (brassidic) acid (24,25) are due to an erroneous interpretation of the greater mobility of *cis*-22:1ω9 versus *cis*-18:1ω9 on $AgNO_3$-TLC (26). Our experience with open tubular GLC and $AgNO_3$-TLC shows only *cis*-monoethylenic isomers in the C_{16}-C_{22} chain lengths.

The chain shortening process in the shorter chain ($\leqslant C_{18}$) monoethylenic fatty acids (see below) is also supported as a general principle by the identification of 16:2ω6 and 14:2ω6 plausibly drived from 18:2ω6, and of 16:3ω3 derived from 18:3ω3. Further shortening of 16:3ω3 would give a *cis*-Δ2,3 bond, apparently not an acceptable result. However, the chain shortening routes 18:1ω9 → 16:1ω9 → 14:1ω9 and 16:1ω7 → 14:1ω7 are acceptable in plants (27), even if better known in animal systems (28). The converse system of a Δ9,10 desaturase acting on 14:0, in lieu of 16:0, would yield 14:1ω5 and then 16:1ω5 by elongation, but this did not extend further to 18:1ω5 (Figs. 1,5). Although we did not observe it, *trans*-16:1ω13 is commonly found in photosynthetic tissue (27) and has been reported in rapeseed oil (29).

Our confirmation of 17:1ω8 (GLC retention basis) was not unexpected, as it is found in various fats and lipids (30), but the *cis* and *trans*-15:1ω10 fatty acids are novel, except for a 15:1ω10 identified in Norway Spruce (*Picea abies*) (31). Although Δ5 desaturases are relatively common in some plant families, leading to a variety of fatty acids with Δ5 unsaturation in seed oils (27,30-32), including (33) *trans*-16:1 and 18:1, there is no reason to expect a C_{15} acid to be singled out as substrate or, alternatively, no reason to associate the Δ5 unsaturation with an ω10 moiety. Since no *trans*-14:1ω9 (Δ5) was found, the *trans*-15:1ω10 (also Δ5) cannot be an artifact formed from *cis*-15:1ω10 during isolation.

An interesting facet of the identification of 14:2ω6 (Fig. 1) was the observation that the component peak size in SILAR-5CP was 30-50% more than that on AP-L, relating both to the 15:0. An explanation readily followed from the identification of *anteiso*-15:0. This acid is coincident with 14:2ω6 on SILAR-5CP but separately identifiable on AP-L (Fig. 3). An old suggestion of varying column temperature (34) would probably permit separation but was not investigated since the more definitive $AgNO_3$-TLC data was available. The formation of *iso*-14:0 and *anteiso*-15:0 presumably follows from acetate addition to a primer of *iso*-4:0 or *anteiso*-5:0 acids originating in amino acid skeletons (35,36). It is remarkable that no higher chain lengths were detected (e.g., *iso*-16:0, *anteiso*-17:0).

The presence of two isomers of 18:3ω3 with one band converted from *cis* to *trans* in three rapeseed oils which had *not* undergone rigorous refining processes such as deodorization was unexpected. It had been concluded that heat and particularly water vapor played a key role in the geometrical conversions (19). The distillation of methyl esters of Tower rapeseed oil fatty acids showed that extended exposure to temperatures of the order of 200 C could cause this isomerization without water vapor. It was even more unexpected to find consistent traces of the A (*cis-9, cis-12, trans*-15-octadecatrienoic acid) isomer of 18:3ω3, with much smaller amounts of what is presumably the C (*trans-9, cis-12, cis-15*-octadecatrienoic acid) isomer in simple seed extracts. The proportions suggest preferential exposure of the Δ15 bond at some stage, possibly at the surface of a membrane or fat globule, to a catalytic material or free enzyme system, while the Δ9 bond would be protected by the bulk of the material. The occurrence of C_{18} conjugated dienoic fatty acids, also at very low levels, has been noted in unprocessed seed oils (C.A. Eaton and R.G. Ackman, unpublished results). Open tubular, gas liquid chromatography is a most suitable tool for researching these trace alterations in fatty acids.

Most of the peaks for shorter chain minor fatty acids discussed in this report are exactly duplicated in other edible seed oils examined in this laboratory. None appear to be metabolically unusual or apt to cause physiological problems.

ACKNOWLEDGMENTS

We acknowledge the assistance of N.F. Crewe for seed extractions and GLC analyses and the cooperation of C.A. Eaton and J.C. Sipos.

REFERENCES

1. Appelqvist, L.-A., in "Rapeseed, Cultivation, Composition, Processing and Utilization," Edited by L.-A. Appelqvist and R. Ohlson, Elsevier, Amsterdam, 1972, p. 124.
2. Ackman, R.G., in "Rapeseed Oil, Meal and By-product Utilization," Edited by J.M. Bell, Rapeseed Association of Canada, Vancouver, 1977, p. 12.
3. Ackman, R.G., JAOCS 43:483 (1966).
4. Beare-Rogers, J., in "Progress in the Chemistry of Fats and Other Lipids," Vol. 15, Edited by R.T. HOlman, Pergamon Press, London, 1977, p. 29.
5. Hilan, H.W., B. Thompson, J.K.G. Kramer, F.D. Sauer, and A.H. Corner, Can. Inst. Food Sci. Technol. J. 10:23 (1977).
6. Vles, R., in "The Role of Fats in Human Nutrition," Edited by A.J. Vergruesen, Academic Press, London, 1975, p. 433.
7. Rocquelin, G., R. Cluzan, N. Vodovar and R. Levillain, Cah. Nut. Diet. 8:103 (1973).
8. Beare-Rogers, J., in "Rapeseed Oil, Meal and By-product Utilization," Edited by J.M. Bell, Rapeseed Association of Canada, Vancouver, 1977, p. 52.
9. Kramer, J.K.G., H.W. Hulan, S. Mahadevan, F.D. Sauer and A.H. Corner, in "Rapeseed Oil, Meal and By-product Utilization," Edited by J.M. Bell, Rapeseed Association of Canada, Vancouver, 1977, p. 64.
10. Ackman, R.G., Lipids, 12:293 (1977).
11. Sebedio, J.-L. and R.G. Ackman, J. Chrom. Sci. 16:204 (1978).
12. Sebedio, J.-L. and R.G. Ackman, Can. J. Chem. 56:2480 (1978).
13. Kuemmel, D.F., JAOCS 41:667 (1964).
14. Haeffner, E.W., Lipids, 5:430 (1970).
15. Ackman, R.G., and S.N. Hooper, J. Chromatog. 86:73 (1973).
16. Ackman, R.G., and S.N. Hooper, Ibid. 86:83 (1973).
17. Ackman, R.G., A. Manzer and J. Joseph, Chromatographia, 7:107 (1974).
18. Ackman, R.G., J. Fish. Res. Bd. Canada 21:247 (1964).
19. Ackman, R.G., S.N. Hooper, and D.L. Hooper, JAOCS 51:42 (1974).
20. Christopherson, S.W., and R.L. Glass, J. Dairy Sci. 52:1289 (1969).
21. Hooper, S.N., and R.G. Ackman, Lipids, 5:288 (1970).
22. Ackman, R.G., J. Chromatogr. Sci. 10:243 (1972).
23. Downey, R.K., Chem. and Ind. (London), May 1, 401 (1976).
24. Lorusso, S., Quad. Merceol., 15(II):189 (1976).

25. Turchetto, E., and S. Lorusso, Nutr. Rep. Int. 15:41 (1977).
26. Ackman, R.G., S.N. Hooper, and J. Hingley, J. Chrom. Sci., 10:430 (1972).
27. Stearns, E.M. Jr., in "Progress in the Chemistry of Fats and Other Lipids," Edited by R.T. Holman, Vol. IX, Part 4, Pergamon, New York, 1970, p. 455.
28. Ackman, R.G., and J.D. Castell, Lipids 1:341 (1966).
29. Homberg, E.E., Phytochemistry, 15:1361 (1976).
30. Pohl, P., and H. Wagner, Fette. Seifen. Anstrichm., 74:424 (1972).
31. Ekman, R. and G. Pensar., Suom. Kemistiseuran Tied. 82:48 (1973).
32. Barve, J.A., F.D. Gunstone, F.R. Jacobsberg and P. Winlow, Chem. Phys. Lipids 8:117 (1972).
33. Bhatty, M.K., and B.M. Craig, Can. J. Biochem. 44:311 (1966).
34. Landowne, R.A., and S.R. Lipsky, Biochim. Biophys. Acta 47:589 (1961).
35. Kates, M., Ann. Rev. Microbiol. 20:13 (1966).
36. Sen Gupta, A.K., Fette Seifen. Anstrichm., 74:693 (1972).

[Received March 7, 1978]