The α,β-Distribution of Oleic, Linoleic and Linolenic Acids in Cruciferae Seed Triglycerides[1]

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ABSTRACT

Correlation studies on lipolysis data from 24 species of Cruciferae seed triglycerides have revealed very regular positional distribution patterns for oleic, linoleic and linolenic acids. When the ratio % 18:1 in β-position/% 18:1 in total triglycerides for each species is plotted vs. the content of Category I acids (16:0, 18:0, plus all C_{20}, C_{22} and C_{24} acids) in the total triglycerides, a smooth curve is obtained. Application of suitable statistical procedures yields a best-fitting curve, from which an equation expressing the % 18:1 in the β-position as a function of the fatty acid composition of the total triglycerides can be derived. The % 18:1 in the α-position is then readily calculated by difference. Similar distinctive relationships have also been developed for linoleic and linolenic acids. Comparison of calculated and experimental results shows that the relationships developed here are considerably more accurate than the previous Gunstone-Mattson and Evans hypotheses for estimating the α,β-distributions of 18:1, 18:2 and 18:3 in Cruciferae seed triglycerides.

INTRODUCTION

The positional distribution of fatty acids in the triglycerides of Cruciferae seed fats was first investigated in 1961 by Mattson and Volpenhein (1). They examined nine species by lipase hydrolysis and found that 16:0, 18:0 and all acids with chain lengths longer than 18 carbons ($>C_{18}$) are esterified almost exclusively at the α-positions. The β-position and the remaining α-positions are occupied by 18:1, 18:2 and 18:3, but no regular distribution patterns were noted for these acids. These results have now been confirmed by other workers (2,3).

The positional distribution of oleic, linoleic and linolenic acids in plant triglycerides has been the subject of continuing research. Trends have been recognized, but clearcut patterns have not been defined. Gunstone (4) and Mattson and Volpenhein (5) have suggested that 18:1, 18:2 and 18:3 are randomly distributed among the free hydroxyl groups remaining after 16:0, 18:0 and all $>C_{18}$ acids are esterified at the α-positions. Further study of lipolysis results by Gunstone et al. (6) and Mattson and Volpenhein (5), however, indicated that among the unsaturated C_{18} acids, oleic and linolenic show a slight preference for the α-positions while linoleic shows a slight preference for the β-position. This was taken into account by Evans et al. (7) when they proposed a new positional distribution hypothesis following three rules: (a) Saturated acids and those with chain lengths greater than 18 carbons are first distributed equally at the two α-positions. (b) Oleic and linolenic acids are then distributed equally and randomly on the unfilled α- and β-positions, with any excess from the α-positions being added to the β-position. (c) All remaining positions are filled by linoleic acid.

Although the Gunstone-Mattson and Evans hypotheses have proven useful in predicting lipolysis data for many common seed fats, they have not proven as accurate in calculating the distribution of 18:1, 18:2 and 18:3 between the α- and β-positions of seed fats such as the Cruciferae, which contain high levels of $>C_{18}$ fatty acids. The Gunstone-Mattson hypothesis, for example, predicts 44 mole % 18:1 at the β-position of radish seed triglycerides vs. 35% found experimentally (1) and 29% 18:2 at the β-position in rapeseed oil vs. 37% ovserved (1). Evans et al. (7) reported consistently large differences between calculated and experimental values for linoleic acids in the Cruciferae and excluded such data during the development of their distribution hypothesis.

A survey of lipolysis data on Cruciferae seed triglycerides indicates that fats of similar $>C_{18}$ content have similar α,β-distributions for 18:1, regardless of the level of 18:1 present. Analogous relationships also exist for 18:2 and 18:3. These observations, together with new lipolysis data, have been used to develop new, more accurate relationships expressing the positional distribution of the C_{18} unsaturated acids in Cruciferae triglycerides as a function of the fatty acid composition of the total triglycerides. These relationships are useful for estimating the α,β-distribution of 18:1, 18:2 and 18:3, and provide an empirical description of the biosynthetic process by which these acids are assembled into triglycerides.

EXPERIMENTAL PROCEDURES

Twenty-seven lipolysis results on 9 genera and 13 species of Cruciferae seed triglycerides have been reported in the literature (1-3,8-12). Since most of these data are for oils of high $>C_{18}$ content, additional Cruciferae genera containing 0-40% $>C_{18}$ acids were examined (Table I) so that all levels of Category I acids would be represented in this study. Data on *Brassica juncea, Brassica napus, Crambe abyssinica, Lunaria annua* and *Sinapus alba* in Reference 2 deviated substantially from the correlations developed below. The validity of these data was therefore checked against lipolysis results on the same species from other laboratories (1,3,9-12) and with new analyses (Table I). These alternative analyses showed good agreement with the observed patterns for all five species; therefore the atypical results from Reference 2 were omitted from the present analysis. Unusual Cruciferae genera (*Lesquerella* and *Cardamine*) containing hydroxy fatty acids in their seed triglycerides have not been included in this study.

Materials

Camelina sativa seeds were provided by F.R. Earle, Northern Regional Research Laboratory, Peoria, Ill. Rapeseed (*B. napus*) containing a normal level of erucic acid were obtained from the Saskatoon Wheat Pool, Saskatoon, Saskatchewan; zero erucic acid rapeseed oil was supplied by B.M. Craig, Prairie Regional Laboratory, Saskatoon, Saskatchewan. Other seeds were purchased from the following suppliers: *Alyssum saxatile* var. *compactum, Arabis alpina* var. *nana compacta, Barbarea vulgaris, Cheiranthus cheiri, Erysimum perofskianum, Hesperis matronalis, Lepidium sativum, Lobularia maritima* and *Malcomia maritima* from Harry E. Saier, Dimondale, Michigan; *Iberis umbellata,*

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[1]Presented in part at the AOCS meeting in San Francisco, California, April 1969.

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TABLE I

Positional Distribution of Fatty Acids in Cruciferae Seed Triglycerides

Cruciferae seed	Analysis	Fatty acids, mole %																	
		14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	20:3	22:0	22:1	22:2	22:3	24:0	24:1	Other
Alyssum saxatile var. *compactum*	TG[a]	4.9	5.6	0.7	1.4	11.3	21.7	52.5	0.5	0.2	0.1	Trace	0.2	---	---	---	---	---	0.9
	β-exp.	0.7	0.5	0.7	0.1	12.6	30.7	54.6	---	0.1	---	---	---	---	---	---	---	---	---
	β-calc.					12.6	31.0	54.5											
Arabis alpina var. *nana compacta*	TG	0.2	5.9	0.3	2.0	12.3	26.9	51.3	0.2	0.2	0.1	---	---	---	---	---	---	---	0.6
	β-exp.	0.4	0.4	0.6	---	14.0	37.5	46.9	---	---	---	---	---	---	---	---	---	---	0.2
	β-calc.					13.4	37.5	49.5											
Barbarea vulgaris	TG	Trace	10.5	0.3	2.5	20.2	9.6	35.6	3.0	10.8	0.6	0.6	0.7	3.9	---	---	0.8	0.9	---
	β-exp.	Trace	0.3	0.1	0.5	22.9	14.3	58.0	0.8	---	0.6	0.6	0.7	---	---	---	1.1	0.1	---
	β-calc.					26.4	16.8	54.0											
Brassica juncea	TG	0.1	2.5	0.3	1.1	10.5	16.7	12.5	0.9	8.4	1.1	0.3	0.9	40.2	1.5	0.3	0.8	1.9	---
	β-exp.	---	0.2	0.4	---	21.3	46.6	30.9	---	0.2	0.1	---	---	0.3	---	---	---	---	---
	β-calc.					22.6	42.7	30.7											
Brassica napus	TG	0.1	2.8	0.2	1.1	17.3	16.3	8.6	0.6	12.0	0.6	0.1	tr	38.7	0.6	0.1	0.1	0.8	---
	β-exp.	0.1	0.3	0.3	---	34.3	42.0	21.6	---	0.8	---	---	---	0.6	---	---	---	---	---
	β-calc.					34.3	40.1	20.2											
B. napus var. zero erucic	TG	Trace	4.4	0.2	1.8	60.5	20.8	9.5	0.5	1.5	0.1	---	0.3	0.2	---	---	0.1	0.1	---
	β-exp.	---	0.3	0.2	---	50.0	33.5	15.9	---	0.1	---	---	---	---	---	---	---	---	---
	β-calc.					65.9	29.0	9.1											
Brassica oleracea var. kohl rabi	TG	Trace	4.3	0.5	0.7	10.4	15.2	13.7	0.4	5.9	0.6	0.2	0.1	44.4	1.2	0.4	0.2	1.6	0.2
	β-exp.	0.1	0.3	0.4	---	23.4	40.1	35.1	---	0.4	---	---	---	0.2	---	---	---	---	---
	β-calc.					22.6	39.0	33.8											
Camelina sativa	TG	0.1	6.5	0.1	2.7	11.8	16.9	36.0	1.9	12.5	2.6	2.1	0.5	4.0	0.2	0.8	0.2	1.1	---
	β-exp.	---	0.3	0.2	---	13.6	30.9	54.7	---	0.3	---	---	---	---	---	---	---	---	---
	β-calc.					15.5	29.9	55.0											
Cheiranthus cheiri	TG	Trace	4.2	0.5	0.8	10.5	17.2	23.7	0.7	7.9	2.0	0.6	1.1	26.8	0.9	0.3	0.5	2.3	---
	β-exp.	---	0.3	0.5	---	18.9	37.5	41.1	---	0.5	0.4	---	---	0.8	---	---	---	---	---
	β-calc.					16.6	36.6	46.8											
Erysimum perofskianum	TG	0.1	3.6	0.8	1.3	13.5	27.0	22.6	0.6	8.4	1.5	0.4	0.3	18.0	0.4	---	Trace	1.5	---
	β-exp.	---	0.4	0.6	0.1	18.5	48.5	31.4	---	0.5	---	---	---	---	---	---	---	---	---
	β-calc.					18.0	48.4	35.5											
Hesperis matronalis	TG	Trace	6.9	1.1	2.3	14.7	25.2	49.3	Trace	Trace	---	---	---	---	---	---	---	---	0.5
	β-exp.	Trace	---	1.3	---	15.0	29.4	54.0	Trace	Trace	---	---	---	---	---	---	---	---	0.3
	β-calc					16.1	35.2	47.9											
Iberis umbellata	TG	0.1	3.0	0.3	0.4	13.0	20.9	5.1	0.2	6.2	0.6	---	---	45.1	0.4	---	---	4.7	---
	β-exp.	0.1	1.6	0.3	---	26.1	52.4	13.2	0.2	1.4	0.3	---	---	3.3	0.4	---	---	0.7	---
	β-calc.					28.8	54.0	12.7											

TABLE I (continued)

Lepidium sativum	TG	0.1	9.0	0.3	2.2	22.7	9.7	34.4	2.3	12.0	0.6	0.7	0.7	3.9	---	---	0.5	0.9	---	
	β-exp.	Trace	0.3	0.3	---	31.7	17.3	50.1	---	0.3	---	---	---	---	---	---	---	---	---	
	β-calc.					29.2	16.7	50.7												
Lobularia maritima	TG	Trace	3.4	Trace	5.1	31.0	7.4	9.1	1.4	41.3	0.6	0.2	0.3	0.2	---	---	---	---	---	---
	β-exp.	---	---	---	---	55.3	16.9	22.6	---	5.2	---	---	---	---	---	---	---	---	---	---
	β-calc.					53.6	16.8	19.5												
Lunaria annua	TG	Trace	1.0	0.1	0.1	26.6	7.4	0.9	Trace	0.8	Trace	---	---	42.0	---	---	---	20.9	0.2	
	β-exp.	---	---	---	---	72.5	20.2	2.4	---	---	---	---	---	0.7	---	---	---	4.2	---	
	β-calc.					72.3	20.4	2.4												
Malcomia maritima	TG	Trace	6.5	0.5	2.1	8.5	17.0	20.1	2.6	23.1	4.0	1.1	0.6	11.7	0.6	0.3	0.4	0.9	---	
	β-exp.	---	0.5	0.6	0.1	15.1	39.9	42.4	---	0.8	0.4	---	---	0.2	---	---	---	---	---	
	β-calc.					15.2	39.5	44.2												
Matthiola incana	TG	0.2	9.1	0.5	3.1	13.3	11.0	62.7	Trace	Trace	---	0.1	---	---	---	---	---	---	---	
	β-exp.	0.1	0.5	0.6	0.1	17.8	16.3	64.6	---	---	---	---	---	---	---	---	---	---	---	
	β-calc.					14.8	15.6	63.7												
Nasturtium officinale	TG	0.1	9.0	0.5	2.2	29.7	23.4	1.8	2.0	10.5	0.9	---	1.5	18.0	---	---	---	0.4	---	
	β-exp.	Trace	0.4	0.2	0.1	47.2	48.3	3.3	---	0.3	Trace	---	---	0.2	---	---	---	---	---	
	β-calc.					44.2	47.3	3.3												

[a] Abbreviations: TG, triglyceride; β-exp., β-position determined experimentally; β-calc., β-position calculated from Equations 3, 5 and 7.

Lunaria annua and *Nasturtium officinale* from W. Atlee Burpee Co., Clinton, Iowa; *Brassica oleracea* var. kohl rabi and *Matthiola incana* from Ferry-Morse Seed Co., Fulton, Ky; and *B. juncea* from Vaughan's Seed Co., Downers Grove, Ill.

Methods

Each seed sample was sorted to remove damaged seeds and foreign material. The seeds were then ground in a Waring Blendor, placed in a paper thimble, and extracted with petroleum ether (30-60 C bp) for 4 hr on a Soxhlet extraction apparatus. The triglycerides were isolated from each seed fat by column chromatography on Florisil (13), and their fatty acid composition was determined by gas chromatography.

The composition of the fatty acids in the β-position of the triglycerides was determined either by lipolysis or by deacylation with Grignard reagent. Hydrolyses with hog pancreatic lipase (General Biochemicals, Chagrin Falls, Ohio) were performed using the procedure of Luddy et al. (14), followed by isolation of the resultant monoglycerides by thin layer chromatography (TLC). Analysis of the fatty acid composition of these monoglycerides directly determined the composition of the β-position. Deacylations with CH_3CH_2MgBr were carried out by the procedure of Brockerhoff et al. (15), followed by isolation of the resultant α,α-diglycerides by TLC. The fatty acid composition of the α,α-diglycerides was determined and then used to calculate the composition of the β-position. Comparison of the two procedures on the same sample showed agreement within 1.5 absolute per cent for minor components (<10%) and within 7 relative % for major components (>10%). This accuracy was considered satisfactory for the present study.

Glyceryl esters were converted into their corresponding methyl esters by KOH-catalyzed methanolysis (16). Fatty acid compositions were determined by gas chromatography of the methyl esters at 175-180 C on 1.82 m x 2.4 mm I.D. columns packed with 10% EGSS-X or 10% EGSS-Y on 100-120 mesh Gas Chrom P (Applied Science Laboratories, State College, Pa.). Peaks were identified by comparison with the elution times of known compounds and by the computational method of Ackman and Burgher (17). All fatty acid compositions are reported in mole per cent.

RESULTS

Oleic Acid

The positional distribution of 18:1 in Cruciferae seed triglycerides exhibits a very regular pattern when suitable parameters are plotted in a graph. The first parameter, the enrichment factor, is a concept originated by Gunstone and Sealy (18) for interpreting lipolysis results by expressing the positional distribution of an acid as a single number. For 18:1, this enrichment factor (E) would be defined as:

$$E_{18:1} = \frac{\text{mole \% 18:1 in } \beta\text{-position}}{\text{mole \% 18:1 in total triglycerides}} = \frac{O\beta}{O_T} \quad [1]$$

The value of $E_{18:1}$ usually varies from 1 to 3 in plant fats, reflecting the enrichment of 18:1 at the β-position. The second parameter is the classification of plant fatty acids into "Category I" and "Category II" acids. This follows the suggestions of Gunstone (4) and Mattson and Volpenhein (5) that the acids found in plant triglycerides fall into two groups: saturated and $>C_{18}$ acids which are almost exclusively esterified at the α-positions (Category I); and 18:1, 18:2 and 18:3 which are found at both the α- and the β-positions (Category II).

Forty-five lipase analyses on 20 genera and 24 species of Cruciferae seed triglycerides are available from the literature and from the present study. The value of $E_{18:1}$ for

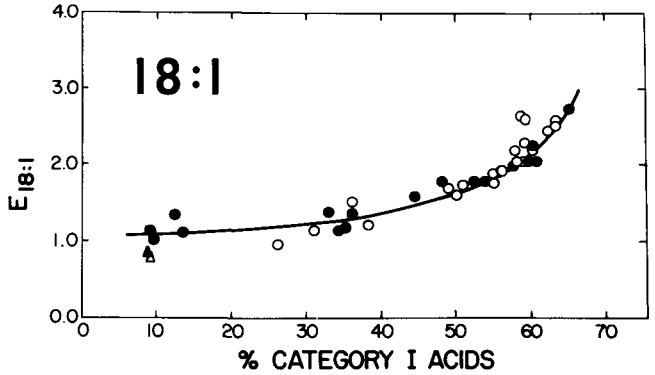


FIG. 1. Relationship between the enrichment factor for oleic acid ($E_{18:1}$) and the content of Category I acids (i.e., 16:0, 18:0 and all C_{20}, C_{22} and C_{24} acids) in Cruciferae seed triglycerides. •,▲ - data from present study. ○,△ - literature data. ▲,△ - zero erucic variety of *B. napus*.

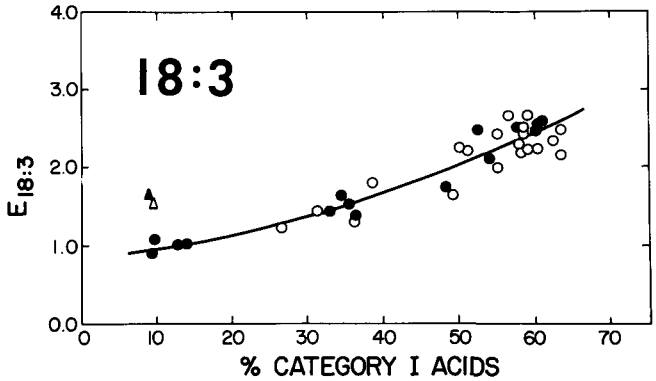


FIG. 3. Relationship between the enrichment factor for linolenic acid ($E_{18:3}$) and the content of Category I acids (i.e., 16:0, 18:0 and all C_{20}, C_{22} and C_{24} acids) in Cruciferae seed triglycerides. •,▲ - data from present study. ○,△ - literature data. ▲,△ - zero erucic variety of *B. napus*.

each sample has been plotted against its content of Category I acids in Figure 1. The data follow a smooth curve with the enrichment factor increasing as the per cent of Category I acids increases. However, the results for the zero erucic variety of *B. napus* (triangular points) fall substantially below the curve in Figure 1; so apparently this hybrid variety deviates from the general pattern.

An equation describing the data in Figure 1 can be derived using the curve fitting procedures outlined by Lewis (19) and Hartley and Booker (20). If C_I is the mole per cent of Category I acids in the total triglycerides, then plotting $1/E_{18:1}$ vs. $1/(100-C_I)$ produces a linear relationship which can be defined by the method of least squares. Translation back into the coordinates of Figure 1 gives the function $E_{18:1} = (100-C_I)/(96-1.3\ C_I)$. A nonlinear least squares computer program (20) is then used to find the precise constants yielding the best-fitting curve. The final equation

$$E_{18:1} = \frac{113 - C_I}{108 - 1.39\ C_I} \qquad [2]$$

describes the experimental data (omitting zero erucic *B. napus*) with a correlation coefficient of 0.96.

Equating [1] and [2] and solving for O_β produces a formula relating the mole % 18:1 at the β-position to the fatty acid composition of the total triglycerides:

$$O_\beta = \frac{O_T\ (113 - C_I)}{108 - 1.39\ C_I} \qquad [3]$$

Formula 3 can now be used to accurately estimate the positional distribution of oleic acid in Cruciferae seed triglycerides. Comparison of the predicted and experimental values shows a standard error of 2.6 absolute per cent for O_β; several comparisons for individual species are listed in Table I. Once O_β is known, O_α is readily calculated in the same manner as with lipolysis data using the equation $O_\alpha = (3O_T - O_\beta)/2$.

Linoleic Acid

The positional distribution of 18:2 in Cruciferae seed triglycerides also shows a regular pattern when its enrichment factor $E_{18:2}$ is plotted against the per cent Category I acids (Fig. 2). $E_{18:2}$ increases as the per cent Category I acids increases, but this relationship is quite different from the one observed for 18:1.

A second degree equation describing the data in Figure 2 can be obtained by applying the method of least squares (19). The best-fitting curve is found to be

$$E_{18:2} = 1.37 - 0.000467\ C_I + 0.000337\ C_I^2 \qquad [4]$$

which gives a correlation coefficient of 0.96 with the experimental data. Substituting L_β/L_T for $E_{18:2}$ and solving for L_β yields an equation relating the per cent 18:2 at the β-position with the fatty acid composition of the total triglycerides

$$L_\beta = L_T[1.37 - 0.000467\ C_I + 0.000337\ C_I^2] \qquad [5]$$

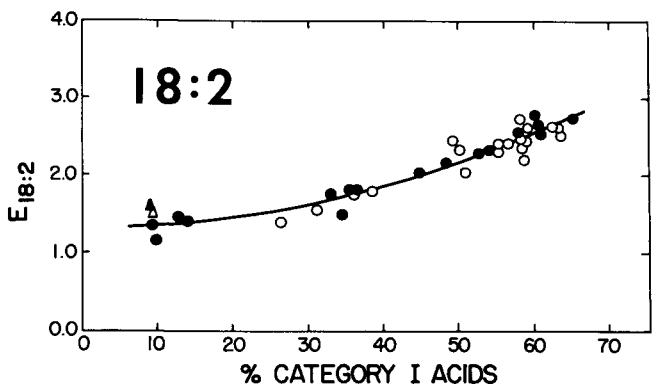


FIG. 2. Relationship between the enrichment factor for linoleic acid ($E_{18:2}$) and the content of Category I acids (i.e., 16:0, 18:0 and all C_{20}, C_{22} and C_{24} acids) in Cruciferae seed triglycerides. •,▲ - data from present study. ○,△ - literature data. ▲,△ - zero erucic variety of *B. napus*.

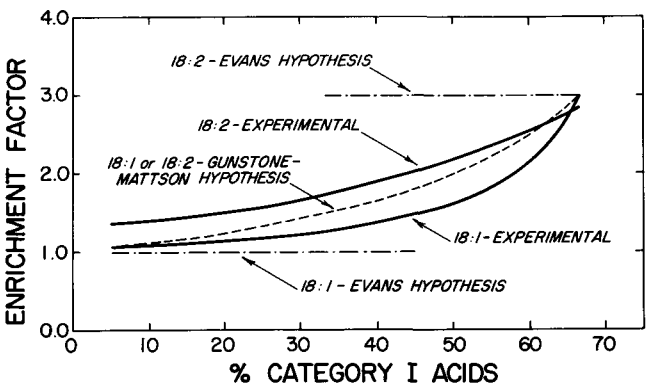


FIG. 4. Graphical comparison of various hypotheses for describing the positional distribution of oleic and linoleic acids in Cruciferae seed triglycerides. Relationship of enrichment factor to the content of Category I acids for: - - - - Gunstone-Mattson hypothesis; — — — Evans hypothesis; —— mathematical models presented here.

Formula 5 now provides an accurate means to estimate the positional distribution of 18:2 as indicated in Table I. Comparison of the predicted and experimental values for L_β shows a standard error of 2.2 absolute per cent. Once L_β is calculated, L_α can be estimated in the same manner as O_α.

Linolenic Acid

When $E_{18:3}$ is plotted vs. the per cent Category I acids (Fig. 3) a consistent trend is again obtained with $E_{18:3}$ increasing as per cent C_I increases. There is a slightly greater scatter in the data points, but this may well reflect greater experimental error in the determination of linolenic acid. Results for 18:3 in the zero erucic variety of *B. napus* (triangular points) again show a marked deviation from the general trend. A second degree equation describing the relationship between $E_{18:3}$ and % C_I can be derived in the same manner as for 18:2. The best fitting curve is

$$E_{18:3}=[0.854+0.00941\ C_I+0.000288\ C_I^2] \quad [6]$$

which describes the experimental data with a correlation coefficient of 0.93. Substituting Ln_β/Ln_T for $E_{18:3}$ and solving for Ln_β yields an equation relating the per cent 18:3 at the β-position to the fatty acid composition of the total triglycerides

$$Ln_\beta= Ln_T[0.854+0.00941\ C_I+0.000288\ C_I^2] \quad [7]$$

and L_α can subsequently be estimated in the usual manner. Equation 7 describes the experimental Ln_β data with a standard error of 2.8%.

DISCUSSION

The positional distributions of 18:1 and 18:2 predicted by the Gunstone-Mattson hypothesis, the Evans hypothesis and the relationships developed here are compared graphically in Figure 4 by plotting $E_{18:1}$ and $E_{18:2}$ vs. C_I. It is immediately evident that the three hypotheses are distinctly different. The Gunstone-Mattson hypothesis assigns oleic and linoleic acids equivalent positional distribution patterns, while the experimental data (Fig. 1 and 2) shows them to be distinctly different. Hence the Gunstone-Mattson hypothesis represents only an average of the 18:1 and 18:2 curves for the Cruciferae and can only be considered an approximation. The Evans hypothesis is not as easy to compare in a graphical presentation, since its values for $E_{18:1}$ and $E_{18:2}$ depend not only on % C_I but also on the per cent 18:2 present. The Evans hypothesis can be treated graphically, however, by dividing Cruciferae seed fats into two categories: Case A, where $E_{18:1}=E_{18:3}>1.00$ (i.e., when excess 18:1 and 18:3 from the α-positions must be added to the β-position); and Case B, where $E_{18:1}=E_{18:3}=1.00$ (i.e., when no excess 18:1 or 18:3 from the α-positions is added to the β-position). $E_{18:2}=3.00$ in Case A, since no 18:2 can be accommodated at the α-positions. Cruciferae seed triglycerides having 33.2% to 66.7% C_I fall into Case A, while samples containing less than 45.1% C_I fall into Case B. There is some overlap of the two cases, depending on the 18:2 content of the triglycerides. Lines for $E_{18:2}=3.00$ in Case A species and for $E_{18:1}=1.00$ in Case B species have been drawn in Figure 4. There is a wide difference between the $E_{18:2}$ values found experimentally (Case A species) and the $E_{18:2}$ values predicted by the Evans hypothesis. The deviation of experimental $E_{18:1}$ values (Case B species) from the Evans prediction is somewhat less but still quite marked.

A similar comparison of hypotheses for the positional distribution of 18:3 in Cruciferae seed triglycerides is shown in Figure 5. Here one finds that the experimental curve for $E_{18:3}$ agrees rather well with the Gunstone-Mattson hypothesis but differs markedly from the Evans proposal.

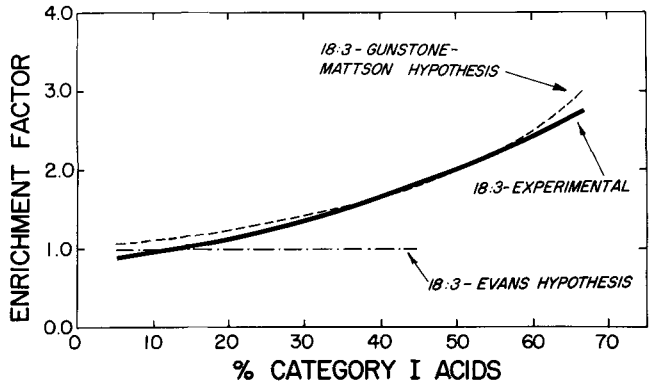


FIG. 5. Graphical comparison of the various hypotheses for describing the positional distribution of linolenic acid in Cruciferae seed triglycerides. Relationship of enrichment factor to the content of Category I acids for: - - - - Gunstone-Mattson hypothesis; — — — Evans hypothesis; —— mathematical model presented here.

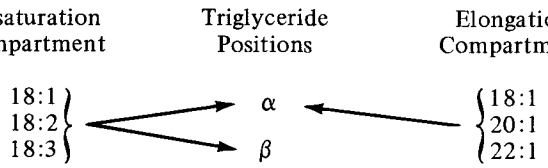
Figures 4 and 5 clearly show, therefore, that the Gunstone-Mattson hypothesis only applies to linolenic acid, while the Evans hypothesis is not generally applicable to Cruciferae seed triglycerides. This latter conclusion confirms the original findings of Evans et al. (7) that their hypothesis was most accurate for non-Cruciferae species. Thus formulas 3, 5 and 7 provide the most accurate method currently available for estimating the positional distribution of 18:1, 18:2 and 18:3 in Cruciferae seed triglycerides. This greater accuracy is undoubtedly the result of deriving the formulas directly from experimental data rather than from model distribution patterns.

There is some evidence that the amounts of individual Category II acids present may have some influence on the value of the enrichment factor. This is noticeable at low values of C_I. The zero erucic variety of *B. napus* has lower values of $E_{18:1}$ and higher values of $E_{18:3}$ than the other species containing 9-14% C_I. The major Category II acid in zero erucic *B. napus* is oleic (52.7-60.5%), while the major Category II acid in the other four low C_I species is linolenic (49.3-62.7%). Perhaps these different levels of 18:1 and 18:3 contribute to the differences found in enrichment values.

The positional distribution patterns described above provide yet additional evidence for the ordered positioning of fatty acids during the biosynthesis of seed triglycerides. Whatever fatty acid mixture is dictated by the genetic characteristics of the species is subsequently esterified to glycerol in a regular and apparently predictable manner. The present study is based solely on lipolysis data and hence only considers the distribution between the β- and the combined α-positions. A single stereospecific analysis on *B. napus* triglycerides (12) indicates that the two α-positions are not equivalent, however; so further regularities in the distribution of fatty acids between the *sn*-1- and *sn*-3-poisitions may become evident when more stereospecific analyses on Cruciferae triglycerides become available.

The biochemical mechanism by which this specific distribution of fatty acids in Cruciferae seed triglycerides is brought about remains to be established. The distinctively different distribution patterns for 18:1, 18:2 and 18:3 may well reflect the varying roles of these acids in the biosynthesis and deposition of fatty acids. Oleic acid has three major functions in Cruciferae seeds: desautration into 18:2 (21,22); elongation into 20:1 and 22:1 (22,23); and direct esterification into triglycerides. Linoleic acid has two major functions: desaturation into 18:3 (21,22), and esterification into triglycerides; while almost all of the 18:3

produced goes directly into triglycerides. For example, the total oleate might possibly be divided between separate elongation and desaturation compartments. Oleate from the elongation compartment might only have access to the α-positions, while 18:1 from the desaturation compartment might have access to both α- and β-positions on the glycerol:



This idea is compatible with the plant breeding experiments of Downey and Craig (23) who found that the levels of 18:1 and 22:1 in *B. napus* seed fat were inversely proportional while 18:2 and 18:3 content remained relatively constant. For the present, however, such an explanation is only speculative.

Attempts to apply formulas 3, 5 and 7 to lipolysis data on 18:1, 18:2 and 18:3 in non-Cruciferae seed fats have shown good agreement in many cases, but also some large discrepancies, especially in fats containing a high level of a single unsaturated acid where a Gunstone-Mattson distribution pattern is most likely. It seems likely, therefore, that the α,β-distributions of 18:1, 18:2 and 18:3 in seed triglycerides are somewhat interdependent. Since the Cruciferae produce only a limited range of 18:1/18:2/18:3 compositions but have a wide range of Category I acid content, positional distribution regularities in their triglycerides are apparently more easily observed. Efforts to adapt the present mathematical models to the interrelationships between levels of the three C_{18} unsaturated acids are currently in progress and hopefully will yield useful relationships applicable to other plant families.

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