TABLE VIII
Glyceride Categories (% mol) of Linoleic-Containing Oils

	"Sat." acids[a] (% mol)	Number of double bonds						
		7 and 6	5	4	3	2	1	0
Safflower	10	47	19	23	8	3	....	....
Tobacco	14	35	17	29	11	8	....	....
J. gossypifolia	14	33	22	32	9	3	1	....
Sunflower	10	14	39	33	11	2	1	....
Maize	14	16	20	31	23	9	1	....
A. mexicana	17	20	18	29	21	10	2	....
Sunflower	7	4	31	36	24	4	1	....
J. multifida	26	10	15	32	24	15	4	....
J. curcas	20	3	16	26	32	19	4	....
Cottonseed	32	11	9	32	22	20	6	....
G. asiatica	33	6	16	29	28	17	4	....
Groundnut	21	....	5	23	40	27	5	....
M. ternifolia	16	....	....	5	55	35	5	....
M. latifolia	48	....	....	6	20	38	35	1

[a] See footnote, Table VI.

of positional distribution. When first elaborated this theory was tested against results then available which were taken from Hilditch's monograph (21). Whilst agreement in the proportion of U_3, US_2, and S_3 glycerides was fair, agreement for U_2S glycerides was less satisfactory and this has been adversely commented upon (22). In Table VI we compare results for these categories of glycerides calculated on the basis of our theory of positional distribution with recent results obtained by ourselves or by others. These show a much better agreement than the earlier results. It should be noted that these comments apply only to vegetable fats.

Polyethenoid Glycerides

In Tables VII and VIII results obtained from our linoleic-containing oils are classified according to the number of polyethenoid acid groups present in the glyceride and the number of double bonds present in the glycerides. The safflower, tobacco, *Jatropha glandulifera*, and the linoleic-rich sunflower seed oil, each with more than 60% of linoleic acid, contain 67–84% of glycerides having two or three linoleic chains and are the only oils listed here which are likely to show drying properties.

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Glyceride Studies. V. The Distribution of Unsaturated Acyl Groups in Vegetable Triglycerides

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Abstract

The distribution of oleic, linoleic, linolenic, petroselinic, hexadec-9 and 11-enoic, sterculic, four conjugated octadecatrienoic acids, isolinolenic, and octadeca-6,9,12,15-tetraenoic acid in vegetable triglycerides has been studied by hydrolysis with pancreatic lipase. The results, discussed in terms of a selectivity factor, indicate that these unsaturated acids do not compete equally for the secondary hydroxyl group.

Introduction

PANCREATIC LIPASE is known to remove acyl groups attached to the two primary glycerol hydroxyls in preference to those attached to the secondary hydroxyl group and lipolysis of vegetable fats by several investigators (1–6) has shown that in most cases 95–100% of the fatty acids in the 2-position are unsaturated C_{18} acids (oleic, linoleic, and linolenic) even when the total content of these acids is as low as 37 or 38% (see reference 7 for a summary of results). This important result has emphasised the non-random character of acyl group distribution in vegetable glycerides and has led to the wide acceptance of the theory of positional distribution (7–9) in place of the earlier ideas of random and widest distribution, neither of which is entirely acceptable. Gunstone (7) and Mattson and Volpenhein (5) have suggested that the acids found in natural triglycerides fall into two groups: those which are preferentially esterified at the 1- and 3- positions (designated "saturated" by Gunstone and Category I acids by Mattson and Volpenhein) and those which are preferentially esterified at the 2-position ("unsaturated" or Category II acids). Mattson and Volpenhein (4) had earlier shown that though oleic, linoleic, and linolenic acids belong to Category II, the C_{20} and C_{22} monoethenoid acids, which characterise the Cruciferae, belong to Category I, behaving like palmitic and stearic acids. From an examination of the distribution of the three unsaturated C_{18} acids these same authors (5) conclude that there is a slight tendency for there to be more linoleic and less oleic in the 2-position than would be expected from their pro-

TABLE I

Enrichment and Selectivity Factors for Acids in Linseed Oil and Morning Glory Seed Oil (4)

	16:0[a]	18:0	16:1	18:1	18:2	18:3	Unsat. acids
Linseed							
Triglyceride (% mol.)	6.1	3.2	0.1	16.6	14.2	59.8	90.7
2-Monoglyceride (% mol.)	0.7	—	0.2	18.8	20.6	59.7	99.3
Enrichment factor	0.11	—	—	1.13	1.45	1.00	1.09
Selectivity factor	—	—	—	1.04	1.33	0.92	—
Morning glory							
Triglyceride (% mol.)	22	12	—	15	42	8	65
2-Monoglyceride (% mol.)	2	1	—	23	64	10	97
Enrichment factor	0.09	0.08	—	1.53	1.52	1.25	1.49
Selectivity factor	—	—	—	1.03	1.02	0.84	—

[a] These figures indicate the number of carbon atoms and double bonds per acid molecule; thus 18:2 represents octadecadienoic acid.

portion in the triglyceride but that otherwise "each of the acids in Category II is randomly distributed among the positions in the triglyceride molecules that are not occupied by Category I acids." We consider the consistent preference of linoleic over oleic acid for the 2-position as significant and we have extended this type of observation to a wider range of unsaturated acids.

Previously we used the concept of an *enrichment factor* (10,11) to discuss acyl group distribution. This is useful when comparing values for acids competing for the 2-position in the same fat, it is less convenient for discussing the behaviour of acids in several different fats and for this reason we now use another term: the *selectivity factor*. The enrichment factor is the ratio of the concentration (molar) of an acid group in the 2-position to its concentration in the total triglyceride. The selectivity factor is the enrichment factor of a particular acid divided by the enrichment factor for all the Category II acids present in the fat under consideration.

This is illustrated by the values for linseed oil detailed in Table I. Enrichment factors are obtained for each acid by dividing the value in the second row of figures by that in the first row. The selectivity factors of the three unsaturated acids result when the individual enrichment factors are divided by the enrichment factor for the unsaturated C_{18} acids taken as a group (1.09). In general we quote enrichment and selectivity factors to one place of decimals; this value may not be very accurate when the proportion of an acid falls below 5% in the triglycerides.

Table I also contains the enrichment and selectivity factors for the same three unsaturated C_{18} acids in Morning Glory seed oil which contains 35% of Category I acids compared with only 9% in linseed oil. The two sets of enrichment factors illustrate the difficulty in comparing these factors in oils with very different proportions of Category I acids. The selectivity factor compensates for these differences and makes

TABLE II

Component Acids (% mol.) of Triglycerides and 2-Monoglycerides

(i) *Gevuina avellana*	16:0	18:0	20:0	22:0	16:1	18:1	18:2	20:1	22:1[a]
1st sample									
Triglyceride	2.3	0.6	1.4	2.0	24.0	43.7	7.8	9.9	8.3
2-Monoglyceride	0.5	—	—	—	26.6	57.3	15.6	—	—
2nd sample									
Triglyceride	2.2	0.7	1.5	1.9	23.9	41.8	8.8	10.3	8.9
2-Monoglyceride	0.4	—	—	—	26.3	54.7	18.6	—	—
(ii) Seed oils containing conjugated trienoic acids									
	16:0	18:0	18:1		18:2	18:3 (conj.)			Position of unsaturation
Tung oil									
Triglyceride	3.1	2.1	11.2		14.6	69.0			9*c*, 11*t*, 13*t*
2-Monoglyceride	0.3	—	8.8		30.5	60.4			
Momordica balsamina									
Triglyceride	9.6	5.3	7.4		9.7	68.0			9*c*, 11*t*, 13*c*
2-Monoglyceride	1.0	—	6.0		20.7	72.3			
Catalpa bignonoides									
Triglyceride	2.3	1.5	9.6		45.1	41.5			9*t*, 11*t*, 13*c*
2-Monoglyceride	0.4	—	14.8		76.2	8.6			
Calendula officinalis									
Triglyceride	3.0	—	4.3		28.8	63.9			8*t*, 10*t*, 12*c*
2-Monoglyceride	0.7	—	4.6		14.4	80.3			
(iii) Seed oils containing isolinolenic and octadecatetraenoic acids									
	16:0	18:0	16:1	18:1	18:2	18:3	iso 18:3	18:4	20:1
Oenothera crocata									
Trigilyceride	4.3	1.3	1.0	75.6	17.8	—	Tr	—	—
2-Monoglyceride	1.7	—	1.1	77.1	16.5	—	3.6	—	—
Oenothera tetraptera									
Triglyceride	14.7	2.0	Tr	5.2	77.4	—	0.7	—	—
2-Monoglyceride	1.6	0.3	0.4	6.9	90.8	—	Tr	—	—
Oenothera biennis									
Triglyceride	9.2	2.3	0.7	12.0	65.8	—	10.0	—	—
2-Monoglyceride	—	0.7	—	15.7	68.1	—	15.5	—	—
Oenothera macrocarpa									
Triglyceride	7.6	1.7	0.5	12.4	66.7	—	11.1	—	—
2-Monoglyceride	—	—	—	15.0	64.5	—	20.5	—	—
Oenothera missouriensis									
Triglyceride	8.5	1.7	—	13.0	65.1	Tr	11.7	—	—
2-Monoglyceride	3.2	1.4	—	21.1	53.6	2.3	18.4	—	—
Forget-me-not									
Triglyceride	11.2	2.0	0.4	33.0	27.6	4.1	14.6	(3.0)	(4.1)
2-Monoglyceride	0.7	—	—	23.1	27.8	4.8	38.0	5.6	—
Blue bur									
Triglyceride	5.9	1.9	0.3	12.8	14.8	35.9	8.7	(17.7)	(2.0)
2-Monoglyceride	0.5	—	0.4	10.7	15.7	27.0	16.9	28.8	—
Borage									
Triglyceride	11.8	4.1	0.3	17.4	37.8	—	21.6	—	4.0[b]
2-Monoglyceride	0.6	—	—	13.7	37.5	—	48.2	—	—
								iso	
Anchusa azurea									
Triglyceride	8.2	1.5	0.8	23.1	32.5	18.4	10.8	(2.5)	(2.2)
2-Monoglyceride	0.7	—	—	16.7	51.9	6.7	17.5	6.5	—
(iv) *Bombacopsis glabra*									
	16:0	18:0	18:1	18:2	Malvalic	Sterculic	Dihydrosterculic		
Triglyceride	53.3	2.7	7.6	4.2	1.6	27.4	3.2		
2-Monoglyceride	5.4	—	16.2	10.7	1.3	62.1	4.3		

[a] See footnote Table I.

[b] Also 22:0, 0.9%; 22:1, 2.1%.

comparison between the two oils easier and more meaningful.

Procedure

We shall discuss results obtained by other workers along with those which we have reported in earlier papers (11–13). Details of experimental procedure are given in these papers but brief notes are added concerning the oils not previously reported. Results are summarised in Table II.

The component acids of the petroselinic acid-containing oils were examined by a new method which will be fully described elsewhere. Oleic and petroselinic esters are not adequately separated by gas liquid chromatography and the proportion of these is determined from the relative amounts of lauric acid (from the Δ^6 isomer) and azelaic acid (from the Δ^9 isomer) produced after oxidation. The results may be slightly less accurate than those obtained in the usual type of fatty acid mixture but are better than earlier results based on the differing solubilities of the lead salts of these two isomeric acids.

Two samples of *Gevuina avellana* were examined. Both contain about 40% of oil and gave about 90% of neutral triglycerides. Our results are quantitatively similar to those previously reported by Cattaneo *et al.* (14) and we have confirmed their observation that the unusual unsaturated acid is hexadec-11-enoic acid. This acid, isolated by a combination of preparative gas liquid chromatography and column chromatography on silica-silver nitrate, gave undecanedioic acid as the only dibasic acid after periodate-permanganate oxidation (15).

When chromatographing esters from oils containing conjugated trienoic acids we observed a tendency for the conjugated esters to appear as two overlapping peaks, perhaps because of isomerisation on the hot chromatographic column (16). Despite this, the combined areas of these two peaks gave a good assessment of the proportion of conjugated esters when checked against the proportion determined by ultraviolet spectroscopy.

Oils containing isolinolenic acid (octadeca-6,9,12-trienoic) present no difficulty because the ester is well resolved from both linoleate and linolenate on a polyester column. The esters of octadecatetraenoic acid and eicosenoic acid, however, overlap on polyester columns and oils containing these acids must also be examined on non-polar (Apiezon L) columns (17). Figures given for these two acids may be slightly less accurate than most of the other values.

Chromatographic analysis of *Bombacopsis glabra* (18) presents difficulties because of the instability of cyclopropene esters (18,19). We isolated the oil with petroleum ether (b.p. 40–60C) at room temperature and prepared the methyl esters by transesterification with sodium methoxide in boiling methanol for five minutes only (20). The esters were examined by gas liquid chromatography on Apiezon L and polyethylene glycol adipate columns before and after hydrogenation in methanol solution in presence of 5% palladium-charcoal.

Discussion

Oleic and Linoleic Acids

Information about 49 seed oils containing oleic and linoleic acid as the only significant unsaturated acids is contained in Table III. These results confirm and extend the views of Mattson and Volpenhein (5). Selectivity factors greater than 1.0 correspond to points lying *above* the "theory" lines drawn in their figures, factors below 1.0 correspond to points *below* the "theory" lines. As the summary at the end of Table III shows linoleic acid almost always has a selectivity factor greater than oleic acid so that the chance of linoleic acid being present in the 2-position is slightly higher and that of oleic acid slightly lower than expected.

TABLE III

Selectivity Factors for Oils Containing Oleic and Linoleic Acid

Ref.	Name	Component Acids (% mol.) "sat."	18:1	18:2[a]	Selectivity Factors 18:1	18:2
13	Sunflower	7	50	42	0.9	1.1
5	Filbert	9	82	8	1.0	1.4
5	Almond	9	70	21	0.8	1.5
5	Sunflower	9	27	64	0.8	1.1
5	Onion	10	26	64	0.9	1.0
13	Safflower	10	13	77	0.9	1.0
13	Sunflower	10	30	60	0.8	1.1
5	Acorn	10	69	20	0.9	1.4
5	Safflower	11	14	75	0.8	1.0
13	Sunflower	12	26	62	0.8	1.1
5	Pecan	12	58	30	0.9	1.2
5	Poppy	12	11	76	0.7	1.0
5	Spinach	13	24	60	0.8	1.1
13	Tobacco	14	14	71	1.0	1.0
11	*Jatropha gossypifolia*	14	17	69	1.0	1.0
5	Corn	14	29	58	0.8	1.1
5	Olive	14	76	8	1.0	1.1
13	Maize	15	30	54	0.9	1.1
1	Olive	15	74	10	1.0	1.0
3	Olive	15	74	11	1.0	0.8
5	Tomato	16	21	61	0.9	1.0
5	Sesame	16	40	44	0.9	1.1
13	*Argemone mexicana*	17	28	55	1.1	1.0
4	Peanut	18	52	27	0.8	1.4
5	Rice	19	41	38	0.9	1.2
13	Groundnut	19	61	18	0.9	1.4
11	*Jatropha curcas*	20	40	37	0.8	1.2
5	Cucumber	21	7	71	0.5	1.1
1	Peanut	21	50	29	0.9	1.2
5	Cashew	22	60	18	0.7	1.9
4	Morro	24	51	22	0.9	1.2
5	Squash	24	16	60	0.8	1.1
11	*Jatropha multifida*	26	23	49	0.8	1.1
4	Marigold	28	9	62	0.8	1.0
5	Cottonseed	28	17	55	0.9	1.0
5	Brazil nut	29	33	39	0.9	1.1
5	Cottonseed	30	17	52	0.9	1.0
1	Cottonseed	30	19	51	0.8	1.1
13	Cottonseed	32	18	50	1.1	1.0
2	Illipé	42	53	5	1.0	0.7
4	Coffee	44	7	48	0.8	1.0
6	Shea	46	47	7	1.0	1.3
2	Palm	46	46	8	1.0	1.2
13	*Madhuca latifolia*	48	38	14	0.9	1.3
1	Palm	49	40	11	1.0	0.9
5	Palm	50	39	9	1.0	1.4
2	Karité	50	45	5	1.0	1.4
6	Palm	51	42	7	1.0	1.1
1	Shea	52	41	6	1.0	1.3

	Selectivity factor <1.0	1.0	>1.0	The larger value
Oleic	32	15	2	5 times
Linoleic	3	14	32	41 times

[a] See footnote, Table I; minor amounts of 16:1 and 18:3 are neglected.

TABLE IV

Selectivity Factors for Oils Containing Linolenic Acid

Ref.	Name	Component acids (% mol.) Sat.	18:1	18:2	18:3	20:1+ 22:1[a]	Selectivity factors 18:1	18:2	18:3
12	Wild rose	4	11	49	36	—	1.0	1.1	0.9
5	Wheat flour	8	27	59	7	—	1.2	1.1	0.8
12	Linseed	9	17	14	60	—	1.0	1.3	0.9
5	Linseed	10	22	15	52	—	1.1	1.4	0.8
12	Candlenut	10	22	37	31	—	1.1	1.3	0.6
5	Walnut	11	15	61	12	—	1.0	1.0	0.9
1	Soya	13	27	52	8	—	1.4	0.9	0.7
5	Soya	16	25	51	8	—	0.7	1.1	0.9
12	Soya	16	24	51	9	—	0.8	1.1	0.8
5	Wheat germ	20	18	55	7	—	0.8	1.1	0.7
4	Morning glory	35	15	42	8	—	1.0	1.0	0.8
4	Mustard	3	16	10	14	56	1.1	1.1	0.9
4	Turnip	4	15	14	12	54	0.9	1.1	1.0
4	Kale	5	22	12	6	54	0.9	1.0	1.2
4	Rape	5	17	17	11	50	0.9	1.1	0.9
4	Rape	6	22	15	14	43	0.9	1.3	0.9
4	Cabbage	7	16	17	12	48	0.8	1.1	1.1
4	*Erysimum perotskianum*	8	13	27	33	28	1.0	1.2	0.9
4	Radish	11	22	15	12	39	0.8	1.2	1.2
4	*Descurania sophia*	12	13	18	38	19	0.8	1.1	1.0

	Selectivity factor <1.0	1.0	>1.0	The largest value
Oleic	10	5	5	Twice
Linoleic	1	3	16	12 times
Linolenic	15	2	3	Once

[a] See footnote, Table I.

TABLE V

Selectivity Factors for Oils Containing Petroselinic Acid

Name	Component acids (% mol.)				Selectivity factors		
	Sat.	18:1(6)	18:1(9)	18:2[a]	18:1(6)	18:1(9)	18:2
Parsley	3	82	4	10	1.0	—	0.9
Carrot	5	73	9	13	0.8	1.5	1.7
Parsnip	5	60	10	24	0.6	1.8	1.8
Caraway	6	47	13	34	1.1	1.2	0.9
Chervil	9	53	3	34	1.6	—	0.2

[a] See footnote, Table I: the figure in parentheses shows the double bond position.

Linolenic Acid

Linolenic acid-containing oils examined by lipolysis fall into two groups. Eleven oils having only C_{18} unsaturated acids are listed in the upper part of Table IV and nine oils in which these unsaturated acids are accompanied by C_{20} and C_{22} monoethenoid acids are listed in the lower part. These longer-chain acids, though unsaturated, behave as Category I acids and have been treated as such in calculating selectivity factors.

In both groups of seed fats linoleic consistently shows a high selectivity factor; 16 out of 20 values exceed 1.0 and only one value is below 1.0. The behaviour of oleic and linolenic acid differs slightly in the two groups of oils. In all the oils having only C_{18}-unsaturated acids, linolenic acid has a selectivity factor below 1.0 (markedly below in most cases), whereas oleic acid shows rather more affinity for the 2-position and has a selectivity factor of 1.0 or more in eight oils, though in most of these it still has a value below that of linoleic acid. In the oils also containing C_{20} and C_{22} acids, linolenic acid shows slightly more affinity for the 2-position competing about equally with oleic acid, but still less effectively than linoleic acid. It seems that in the biosynthesis of vegetable triglycerides linoleic acid is slightly more effective in acylating the secondary hydroxyl group than either oleic or linolenic acid.

Petroselinic Acid

Petroselinic acid (octadec-6-enoic) is an isomer of oleic acid characteristic of the Umbelliferae where it occurs along with oleic and linoleic acids and minor amounts of saturated acids. Our lipolysis studies (Table V) show that petroselinic acid accompanies oleic and linoleic in the 2-position but the selectivity factors for the three unsaturated acids in the five oils examined show rather more variation than in the linoleic and linolenic containing oils. Chervil seed oil seems to be unusual in that its linoleic acid shows a remarkably low selectivity factor and we have confirmed that the octadecadienoic acid is the 9*c*, 12*c* isomer.

Hexadecenoic Acids

Though hexadecenoic acid rarely exceeds 1 or 2% of the component acids, it is a major component of a few seed oils. The results for avocado have previously been given by Mattson and Volpenhein (5) and we have now examined two samples of *Macadamia ternifolia* seed oil and two of *Gevuina avellana* seed oil with the results given in Table VI. The behaviour of oleic and linoleic acid is normal in that oleic has a selectivity factor close to 1.0 and linoleic has a higher factor. Hexadecenoic acid, whether the Δ^9 acid in avocado or *M. ternifolia* or the Δ^{11} acid in *G. avellana*, occurs in the 2-position but its low selectivity factor shows that it is less likely to be found there than either of the C_{18} unsaturated acids which accompany it.

TABLE VI

Selectivity Factors for Oils Containing Hexadecenoic Acid

Ref.	Name	Component acids (% mol.)				Selectivity factors		
		"Sat."	16:1	18:1	18:2[a]	16:1	18:1	18:2
5	Avocado	37	20	27	14	0.4	1.0	1.8
13	*Macadamia ternifolia*	18	27	52	3	0.7	1.1	—
		23	18	56	3	0.7	1.1	—
*	*Gevuina avellana*	24	24	44	8	0.8	1.0	1.5
		25	24	42	9	0.8	1.0	1.6

[a] See footnote, Table 1.
* Present work.

TABLE VII

Selectivity Factors for Oils Containing Conjugated C_{18}-Trienoic Acids

		Component acids (% mol.)				Selectivity factors		
		Sat.	18:1	18:2	conj. 18:3[a]	18:1	18:2	conj. 18:3
Tung	9*c*, 11*t*, 13*t*	5	11	15	69	0.8	2.0	0.8
Momordica balsamina	9*c*, 11*t*, 13*c*	15	7	10	68	0.7	1.8	0.9
Catalpa bignonoides	9*t*, 11*t*, 13*c*	4	10	45	41	0.9	1.0	—[b]
Calendula officinalis	8*t*, 10*t*, 12*c*	3	4	29	64	1.1	0.5	1.2

[a] See footnote, Table 1.
[b] Considered to be a Category I acid (see text).

Conjugated Octadecatrienoic Acids

Elaeostearic acid (9*c*, 11*t*, 13*t*) is the best known of the C_{18} conjugated trienoic acids but recent work, particularly by Hopkins, has shown the occurrence of several configurational and positional isomers. We have been able to examine three of these in addition to elaeostearic acid: the 9*c*, 11*t*, 13*c* isomer in *Momordica balsamina* (21), the 9*t*, 11*t*, 13*c* isomer in *Catalpa bignonoides* (22), and the 8*t*, 10*t*, 12*c* isomer in *Calendula officinalis* (23).

These four isomeric acids show remarkable differences in their selectivity factors. Tung oil and *M. balsamina* seed oil, though containing different acids, are fairly similar; the conjugated triene acid acylates the secondary hydroxyl with about the same efficiency as oleic acid but much less effectively than linoleic acid which shows very high factors (2.0 and 1.8). The triene acid (catalpic) in *C. bignonoides* (9*t*, 11*t*, 13*c*), on the other hand, behaves as a Category I acid. The triglycerides contain 42% of this acid but the 2-monoglyceride contains only 9% and the enrichment factor (0.2) is of the same order as is commonly attained by palmitic acid in many seed fats. The selectivity factors for oleic and linoleic acid (Table VII) are therefore calculated on the basis that the trienoic acid belongs to Category I. *C. officinalis* seed oil, containing a C_{18} acid with the same configuration as catalpic but in the 8, 10, 12 rather than the 9, 11, 13 positions is different again. The conjugated acid and oleic acid have high selectivity factors whilst linoleic has an unusually low value. Because of this we isolated the C_{18}-dienoic acid and proved it to be the 9*c*, 12*c* isomer. Another illustration of the unusual behaviour of these conjugated C_{18} trienoic acids is found in the lipolytic behaviour of *M. charantia* seed oil (24); glyceride composition calculated from lipolysis results does not agree with that obtained by Young's oxidation procedure.

Isolinolenic and Octadecatetraenoic Acids

The *Oenothera* genus is unusual in that the seed fats of some, but not all, species contain isolinolenic acid (octadeca-6,9,12-trienoic); we found this acid in three of the five species examined. Some Boraginaceae species have recently been shown (17,25,26) to contain in their seed oils, in addition to oleic and linoleic acid,

TABLE VIII

Selectivity Factors for Oils Containing Isolinolenic and Octadecatetraenoic Acids

	Component acids (% mol.)						Selectivity factors				
	"Sat."	18:1	18:2	18:3	iso 18:3	18:4[a]	18:1	18:2	18:3	iso 18:3	18:4
Oenothera crocata	6	76	18	—	—	—	1.0	0.9	—	—	—
O. tetraptera	17	5	77	—	1	—	1.2	1.0	—	—	—
O. biennis	12	12	66	—	10	—	1.2	0.9	—	1.4	—
O. macrocarpa	9	12	67	—	11	—	1.1	0.9	—	1.7	—
O. missouriensis	10	13	65	—	12	—	1.5	0.8	—	1.5	—
Forget-me-not	17	33	28	4	15	3	0.6	0.8	—	2.1	(1.7)
Blue bur	10	13	15	35	9	18	0.8	1.0	0.7	1.7	(1.5)
Borage	23	17	38	—	22	—	0.6	0.8	—	1.7	—
Anchusa azurea	12	23	32	18	11	3	0.8	1.4	0.6	1.4	(2.1)

[a] See footnote, Table 1.

linolenic acid and/or isolinolenic acid and/or octadeca-6,9,12,15-tetraenoic acid and we have been able to examine four of these seed oils.

The results from the *Oenothera* oils are unusual in that oleic acid has, surprisingly and consistently, a higher selectivity factor than linoleic acid and, even more interesting, isolinolenic acid shows a very high selectivity factor. In the Boraginaceae oleic and linoleic fall into their more customary order though the values tend to be lower than usual. In contrast, the factors for isolinolenic acid and (less certainly) for octadecatetraenoic acid are unusually high. The seven selectivity factors for isolinolenic acid lie between 1.4 and 2.0. This means that the proportion of this acid in the 2-position is 40–100% higher than would be expected if all Category II acids were statistically distributed at this position. This high value is in marked contrast to the low factor associated with the more common isomeric form of linolenic acid.

Sterculic Acid

Based on a single result for *Bombacopsis glabra* seed oil sterculic acid [8-(2′-*n*-octylcycloprop-1′-enyl)-octanoic] seems to take its place along with oleic and linoleic acid in the 2-position. However it should be noted that oleic and linoleic acid comprise only 12% of the total acids and cannot therefore fill all the 2-position, and that the total content of unsaturated acids is only 41%.

General Comments

Our results must be considered only as a preliminary communication. They provide interesting evidence about the distribution of acyl groups between the secondary and the two primary hydroxyl groups of glycerol but they need to be supported by more information before any very definite conclusions are obtained. We plan to do this in two directions: we hope to examine more seed oils containing the acids we have discussed in this paper and we hope to extend the range of acids. In view of the size of this task and the difficulty of obtaining samples we hope that others will also direct their attention to this problem and that lipolysis data can be expressed in similar terms to ours.

Obviously it is inadequate to think of acids as merely falling into two groups each member of which behaves in identical manner. It has been known for some time that Category I acids acylate the 2-position to a minor extent and do so entirely when Category II acids are absent or present in very small amount. It is now apparent that Category II acids vary in their ability to acylate the 2-position; some tend to get more than their statistical share of the secondary hydroxyl groups, others get rather less. The acids which have been examined so far can be listed in the following order:

Category II	Selectivity factor
(i) Isolinolenic, octadecatetraenoic	1.4–2.0
(ii) Linoleic, 18:3(8*t*, 10*t*, 12*c*)	1.0–1.2
(iii) Oleic, petroselinic, linolenic, sterculic 18:3 (9*c*, 11*t*, 13*t*), 18:3(9*c*, 11*t*, 13*c*)	0.8–1.0
(iv) Hexadecenoic	0.7–0.8
Category I	**Enrichment factor**
Eicosenoic, docosenoic, palmitic, stearic, 18:3 (9*t*, 11*t*, 13*c*)	$\ngtr 0.2$

This must be considered as a provisional sequence which may need considerable modification as further results become available. It is difficult at this stage to see a pattern in these results, especially in the widely differing results obtained with the conjugated trienoic acids, but it is interesting that two of the acids in group (i) and (ii) are essential fatty acids (linoleic and isolinolenic) and that the conjugated trienoic acid also contains the end group, $CH_3 \cdot [CH_2]_4 \cdot CH =$ (*cis*), characteristic of the linoleic family.

Finally it must be acknowledged that these results depend on the reliability of the enzymatic hydrolysis procedure. There is plenty of evidence that for the *normal range of saturated and unsaturated acids* pancreatic lipase is primarily position-specific (showing a strong preference for the 1- and 3- positions) and only to a very minor extent structure-specific, i.e. depending on the structure of the glyceridic acyl group. It is not possible to say with certainty that this behaviour will extend to the less common unsaturated acids and further work is required to check this.

TABLE IX

Selectivity Factor for an Oil Containing Sterculic Acid

	Component acids (% mol)				Selectivity factors		
	"Sat."	18:1	18:2[a]	sterculic	18:1	18:2	sterculic
B. glabra	56	8	4	27	1.0	1.2	1.1

[a] See footnote, Table 1.

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The Triglyceride Composition of Linseed Oil

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Abstract

The triglyceride composition of linseed oils obtained under different ecological conditions and having different fatty acid compositions was determined by a combination of several chromatographic techniques. The triglyceride mixture was first separated in 8 fractions of different polarity by reversed-phase paper chromatography. Each glyceride fraction was then separated in a partition chromatographic system as the triglyceride coordination complexes with silver ions into individual compounds. The fatty acid compositions of the original oil, single glyceride fractions, and individual triglycerides were determined by gas-liquid chromatography. The molar ratio between the two neighboring glyceride fractions was determined by relating the fatty acid composition of each fraction to the fatty acid composition of their sum. The triglyceride composition of the total oil was then calculated from these results.

The presence of 18–19 triglycerides was ascertained in the samples studied, and the molar concentration of each glyceride was estimated. Linseed oil contains only triunsaturated and monosaturated-diunsaturated triglycerides. Within each of these types the fatty acid distribution is close to random. At the same time, the content of some triglycerides departed regularly from a random pattern.

A method for calculation of linseed oil triglyceride composition from the fatty acid composition is given.

The same general pattern of glyceride formation in linseed is followed regardless of ecological conditions; therefore, the qualitative and quantitative triglyceride composition reflects the differences in fatty acid composition of linseed oil.

Introduction

PREVIOUS CONTRIBUTIONS from our laboratory (1,2) have established the triglyceride composition of poppyseed and cottonseed oils. These oils are rather simple in their major fatty acid composition, containing only palmitic, stearic, oleic and linoleic acids. However, most natural fats are much more complex in their fatty acid composition, and the quantitative determination of the triglyceride composition of these fats involves considerable difficulty in spite of modern separation techniques.

Of all vegetable oils containing five major fatty acids, linseed oil has the greatest practical importance. Consequently, the glyceride composition of this oil has been studied by many workers. The qualitative and quantitative triglyceride composition of linseed oil has been investigated by crystallization of brominated glycerides (3), adsorption chromatography on an alumina column (4), fractional crystallization from acetone followed by ester distillation (5), countercurrent distribution (6), reversed-phase paper chromatography (7), selective hydrolysis by pancreatic lipase (8,9), and partition chromatography on a vulcanized rubber column (10,11).

As a result of these studies, some glycerides were identified and their amounts estimated (3,4,6). Also the quantities of the various triglyceride types present (triunsaturated, monosaturated-diunsaturated, etc.) and the number of various glyceride fractions of different polarity were determined (7–11). Nevertheless, the triglyceride composition of linseed oil has not yet been fully characterized.

The present work was undertaken to investigate the triglycerides of two linseed oils of different fatty acid composition by reversed-phase partition chromatography, partition chromatography in a silver nitrate-containing system, and gas-liquid chromatography (1,2,12–14). It was found that the specific distribution of fatty acids among glycerides and the individual triglyceride content of the oil is markedly influenced by the genotype and phenotype characteristics of the linseed plants.

Experimental

Material

Linseed plants of the Krupnosemyanny-3 variety were grown in 1961 on a field plot at this Institute. A part of the harvested seed was sown out again on the same plot in the spring of 1962. In 1962, the development of plants was retarded considerably by the cold and rainy summer. The weather conditions of the growing seasons and the cultivation of the experimental plants have been described in detail earlier (15). The oil was obtained by cold expression. The oil content of seeds was evaluated by refractometery (15). The iodine value of the oil was determined by the Hanus method (1).

Methods

The triglycerides were resolved into separate fractions of different polarity by reversed-phase paper partition chromatography (1,2,13) using an acetone: acetic acid (1:1)/aliphatic hydrocarbons (bp 260–310C) system. For the separation of the more polar glyceride fractions we used dodecane instead of the high-boiling hydrocarbons. The triglyceride fractions were identified by their polarity constant K_2 and relative chromatographic mobility R_2 (12). The fractions were then eluted from the chromatograms with n-hexane (1,2) and converted into fatty acid methyl esters. The fatty acid compositions of single fractions and of the total oil were determined by gas-liquid