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STEREOSPECIFIC ANALYSIS OF SOME CRUCIFERAE SPECIES

B. Töregård and O. Podlaha

Introduction

If the two primary carbon atoms in the glycerol moieties in a triacylglycerol are linked to different fatty acids, a centre of asymmetry is created. The three positions in a triacylglycerol are thus stereochemically unequal, as can be seen in figure 1. The goal of stereospecific analysis is to determine how the fatty acids are distributed over the three positions. The most commonly used nomenclature for numbering the positions is based on a standard Fischer projection of glycerol with the middle hydroxyl group to the left of the carbon chain. The carbon atoms are then numbered in top to bottom sequence. The prefix sn-(sterospecifically numbered) is placed before the name of the compound.

Figure 1:

sn-nomenclature for triacylglycerols

$CH_3O.OR_1$	sn-1-position
$RO.\overset{*}{C}H$	sn-2-position
$CH_3O.OR_2$	sn-3-position

R_* = arbitrary acyl group
C* = asymmetric carbon atom

Procedures for stereospecific analysis of triacylglycerols have been available since 1965 (BROCKERHOFF, 1965, 1967). Previously, a restricted positional analysis had been used, which could only distinguish between the sn-2- and the combined sn-1- and sn-3-positions (ß- and α -positions) (LUDDY et al., 1964; MATTSON and BECK, 1956; MATTSON and VOLPENHEIM, 1968).

Several results from analyses of triacylglycerol of Cruciferae species with respect to sn-2- and the combined sn-1-, -3-positions have been published (MATTSON and VOLPENHEIM, 1968; EVANS et al. ; LITCHFIELD, 1971). The fatty acid distribution between the sn-1- and sn-3-positions has however only been investigated by BROCKERHOFF and YURKOWSKI (1966) and by PODLAHA and TÖREGARD. BROCKERHOFF and YURKOWSKI analysed a single sample of Brassica napus, while PODLAHA and TÖREGARD recently studied four different samples of Brassica napus and campestris. The results showed a clearly non-random distribution between the sn-1- and sn-3-positions for most of the major acids.

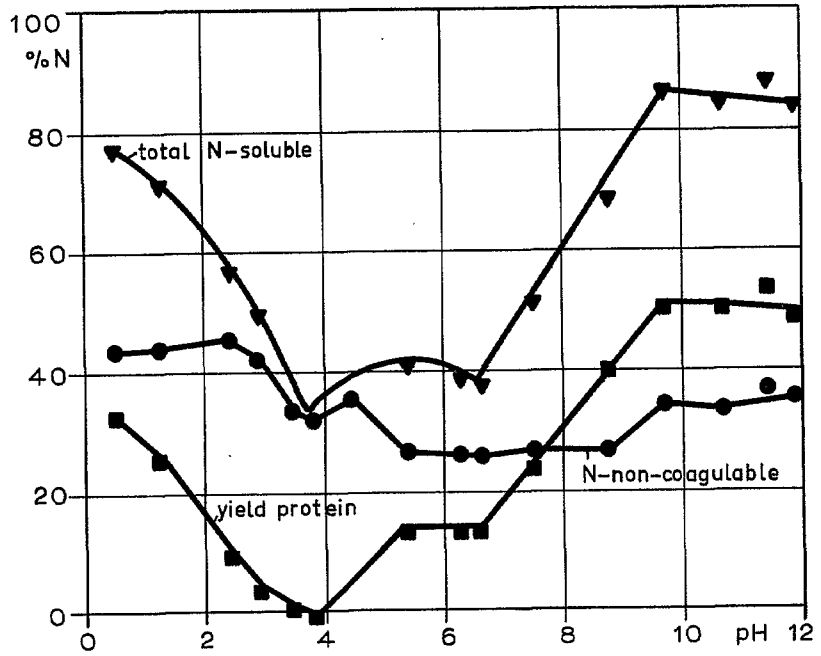
This paper presents further results from stereospecific analysis of Cruciferae species.

Materials and methods

The seeds were obtained from the Swedish Seed Association, Svalöf. The oil was cold pressed from the seeds and the triacylglycerol fraction isola-

ted by chromatography on a silicic acid column. Seed materials and the fatty acid composition of the triacylglycerols are apparent from table 1.

The stereospecific analysis were performed principally according to BROCKERHOFF's procedure in the version of CHRISTIE and MOOR (1969) and ÅKESSON (1969). The fundamental steps are shown in figure 2.



First the triacylglycerols are deacylated in a completely random manner with a Grignard reagent (ethyl magnesium bromide). The resulting sn-1, 2(2, 3)-diglycerides are isolated by preparative TLC and reacted with phenyl dichlorophosphate to produce a mixture of the phosphatides sn-1, 2-diacyl-3-phosphatidylphenol and sn-2, 3-diacyl-1-phosphatidylphenol. The phosphatides are then hydrolyzed by the enzyme Phospholipase A, which only liberates the fatty acids from the 2-position of the sn-3-phosphatides, but leaves the sn-1-phosphatides unhydrolyzed. After the separation by TLC and transesterification to methyl esters, the lysophosphatide fraction is analyzed by gas chromatography, giving the distribution of fatty acids in the sn-1-position. The composition in position sn-2 is determined by a pancreatic lipase hydrolysis, which specifically hydrolyzes the sn-1- and sn-3-positions in triacylglycerols. The composition in position 3 is deter-

Table 1: Fatty acid composition in triglycerides of the Cruciferae species studied

SPECIES and VARIETY	FATTY ACID COMPOSITION (MOLE %)												
	16:0	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1	22:2	24:0	24:1
B. napus ORO	4.8	1.2	62.6	18.9	9.5	-	1.6	0.1	-	0.6	-	-	0.2
B. napus SINUS	5.0	1.0	45.2	18.1	11.1	-	8.0	0.2	0.2	10.7	0.1	0.1	0.3
B. napus SV 71-6	4.2	1.3	29.0	17.2	10.9	0.5	14.0	0.5	0.1	21.5	0.1	-	0.4
B. campestris BELE	2.6	0.8	27.8	16.7	9.9	-	11.2	0.5	0.4	27.8	0.5	0.2	1.1
B. napus GULLE	4.4	0.9	19.6	15.2	10.8	0.2	13.8	0.6	0.2	33.6	0.5	-	0.6
B. campestris DURO	2.2	0.8	15.6	14.5	10.6	0.6	10.1	0.7	0.5	43.4	0.5	-	0.8
B. napus PANTER	3.3	0.7	11.7	13.7	10.6	-	7.1	0.5	-	50.2	1.1	-	0.8
Sin. alba TRICO	3.4	0.9	26.8	9.1	11.9	-	9.7	0.4	0.6	35.2	0.3	0.4	1.9
Crambe abyssinica	2.1	0.6	17.4	9.2	7.7	0.7	2.5	0.1	1.4	56.6	0.7	0.2	0.7

mined indirectly by calculation from the fatty acid composition in the original triacylglycerols and in the sn-1- and sn-2-positions, or from the analysis of the uncleaved 2,3-diacyl-phosphatides and that of position 2.

Results and discussion

Table 2 gives results from the analysis of a synthetic triacylglycerol, sn-1,2-dipalmito-3-myristin. If we assume that the triacylglycerol has the theoretical composition, the figure indicates that the method is slightly inaccurate, especially for the sn-1-position. The precision is fair, and

Table 2: Stereospecific analysis of 1,2-dipalmitoyl-3-myristoyl-glycerol

	C 14 : 0			C 16 : 0		
	Mol %	Theor.	s	Mol %	Theor.	s
sn-1-position	3.7	0	0.5	96.3	100	0.5
sn-2-position	1.9	0	0.2	98.1	100	0.2
sn-3-position	99.5	100	0.5	0.5	0	2.0

on the whole the complicated and laborious method can be regarded as satisfactory.

Results from the stereospecific analysis of the oils investigated are presented in table 3. The analyses were checked by calculating the fatty acid composition in position sn-3 in two independent ways (from 1-acyl-3-lysophosphatidylphenols and from 2,3-diacyl-1-lysophosphatidylphenols). Results, which showed greater differences than 2 mol % were rejected.

Figures 3 - 8 show the distribution pattern for the major fatty acids from the natural triacylglycerol mixture as a function of the erucic acid content. The y-axis on the diagrams represents the percentage distribution in moles of the fatty acids among the three positions. Thus a random distribution of an acid is indicated by 33.3 % in all three positions. More than 33.3 % means an enrichment in that particular position, and less than 33.3 % implies the opposite. Symbols in parentheses indicate the non-Brassica species.

Figure 3 shows the well-known fact that the saturated palmitic and stearic acids are strongly accumulated in the outer positions. No significant difference between the sn-1- and sn-3-positions can be detected because of the relatively large error in the method for such small components as C16:0 and C18:0.

Gadoleic acid has the same strong preference for the outer positions (Fig. 4). However, between the outer positions there appears to be a systematic distribution pattern, in that the preference for sn-3 is changed to a preference for sn-1 when the erucic acid content rises above about 30 %.

Table 3: Stereospecific analysis of the Cruciferae species studied

SAMPLE (variety)	POS.	FATTY ACID COMPOSITION (MOLE %)											
		16:0	18:0	18:1	18:2	18:3	20:0	20:1	20:2	22:0	22:1	22:2	24:0	24:1
ORO	1	6.4	1.7	64.7	15.8	7.4	0.2	1.8	-	0.2	0.7	-	-	0.2
	2	0.2	0.1	52.7	30.6	15.7	-	0.3	-	-	0.1	-	-	-
	3	7.8	1.8	70.4	10.3	5.4	-	2.7	0.3	0.7	1.0	-	-	0.4
SINUS	1	6.6	1.5	48.3	12.5	6.5	0.1	10.4	0.3	0.1	12.4	0.1	-	0.3
	2	0.2	0.1	43.3	34.6	21.4	-	0.1	-	-	-	-	-	-
	3	8.2	1.4	44.0	7.2	5.4	-	13.5	0.3	0.5	19.7	0.2	-	0.6
SV 71-6	1	6.1	1.5	30.2	9.9	5.9	0.1	17.2	1.0	0.1	25.4	0.3	-	0.8
	2	0.2	-	38.2	36.3	23.8	-	0.3	0.1	-	0.2	-	-	-
	3	6.3	2.4	18.6	5.4	3.0	1.4	24.5	0.4	0.2	38.9	-	-	0.4
BELE	1	4.3	1.7	35.2	13.4	7.0	0.2	16.5	1.1	0.1	18.5	0.2	-	0.6
	2	0.2	-	41.7	35.8	20.8	-	0.6	0.1	-	0.3	-	-	-
	3	3.3	0.7	6.5	0.9	1.9	-	16.8	0.3	1.1	64.6	1.3	0.6	2.7
GULLE	1	5.6	1.5	19.2	4.9	3.2	0.2	20.0	1.2	0.2	42.3	0.6	-	0.8
	2	0.2	0.1	32.9	38.9	27.5	-	0.3	-	-	0.2	-	-	-
	3	7.4	1.1	6.7	1.8	1.7	0.6	21.1	0.6	0.6	58.3	0.9	-	1.0
DURO	1	2.9	1.5	13.5	3.1	2.2	0.4	18.0	1.5	0.6	52.9	0.9	0.1	1.6
	2	0.2	-	32.0	39.3	27.3	-	0,4	0.1	-	0.3	-	-	-
	3	3.5	0.9	1.3	1.1	2.3	1.4	11.9	0.5	0.9	77.0	0.6	-	0.8
PANTER	1	4.7	1.5	3.7	1.2	0.7	-	12.9	1.0	-	70.1	1.3	-	1.3
	2	0.1	-	29.9	39.2	30.1	-	0.3	0.1	-	0.3	-	-	-
	3	5.1	0.6	1.5	0.7	1.0	-	8.1	0.4	-	80.2	2.0	-	1.1
SINAPIS ALBA	1	5.9	1.9	26.9	4.4	3.9	0.4	17.4	0.5	0.2	35.0	0.2	0.1	1.7
	2	0.3	-	49.5	21.6	26.8	-	0.5	0.1	-	0.7	-	-	-
	3	4.0	0.8	4.0	1.3	5.0	-	11.2	0.6	1.6	69.9	0.7	1.1	4.0
CRAMBE ABYSSINICA	1	2.3	1.3	1.3	0.5	0.3	0.6	4.2	0.2	1.5	83.0	1.0	0.5	2.2
	2	0.5	0.1	50.0	26.0	20.8	-	0.5	-	-	1.5	-	-	-
	3	3.5	0.4	0.9	1.1	2.0	1.5	2.8	0.1	2.7	85.3	1.1	0.1	-

Figure 3:

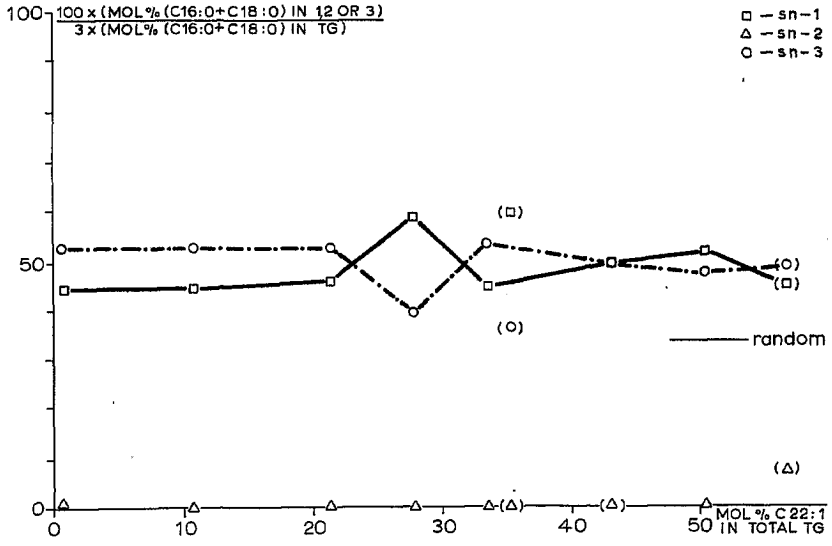


Figure 4:

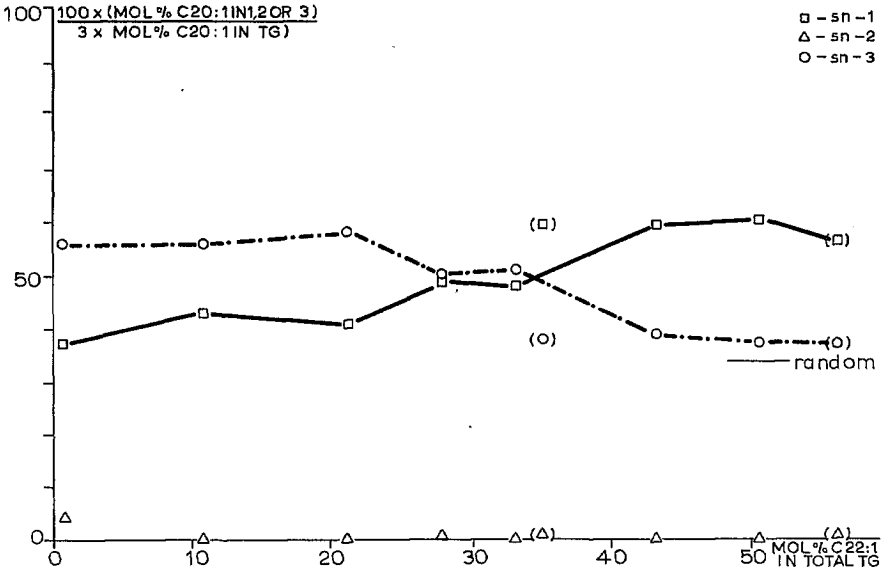


Figure 5:

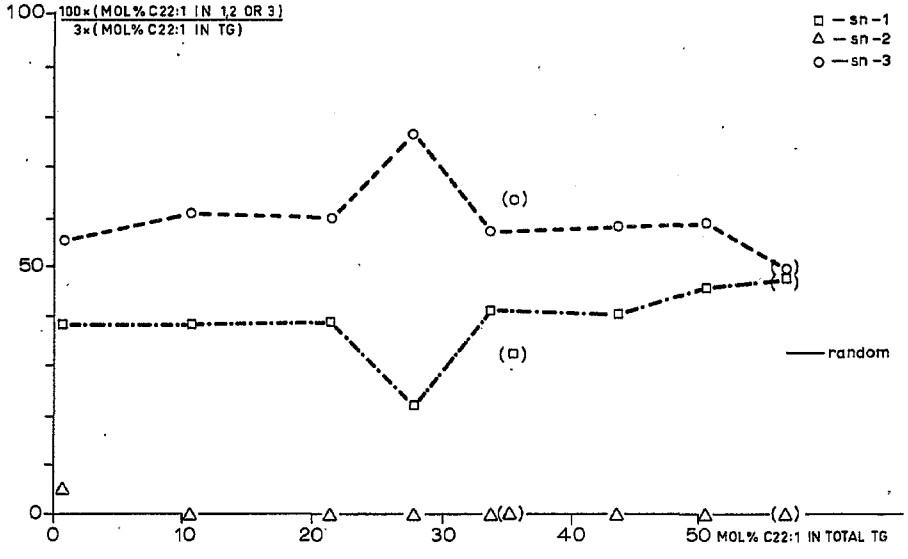


Figure 5 gives the distribution of erucic acid. Again there is a nearly complete concentration to the outer positions with the exception of the very low erucic acid species. The concentration to the sn-3-positions is clearly evident for the Brassica and Sinapis species, while for Crambe the distribution is symmetrical between sn-1 and sn-3. Palmitic and stearic acids, as well as gadoleic and erucic acids, are called the Category I-acids, indicating that they are first esterified to the outer positions (LITCHFIELD, 1971). The unsaturated C18-acids, the Category II-acids, are then distributed on the remaining positions.

As can be seen from figure 6, oleic acid is concentrated to the inner position. The distribution between the sn-1- and sn-3-positions is non-random and correlated to the erucic acid content. Oleic acid begins to be unequally distributed between sn-1 and sn-3 with erucic acid contents above 10 %, and then again becomes fairly equally distributed when the erucic acid reaches the highest levels. The sn-1-position is preferred, in some instances very strongly.

Figure 6:

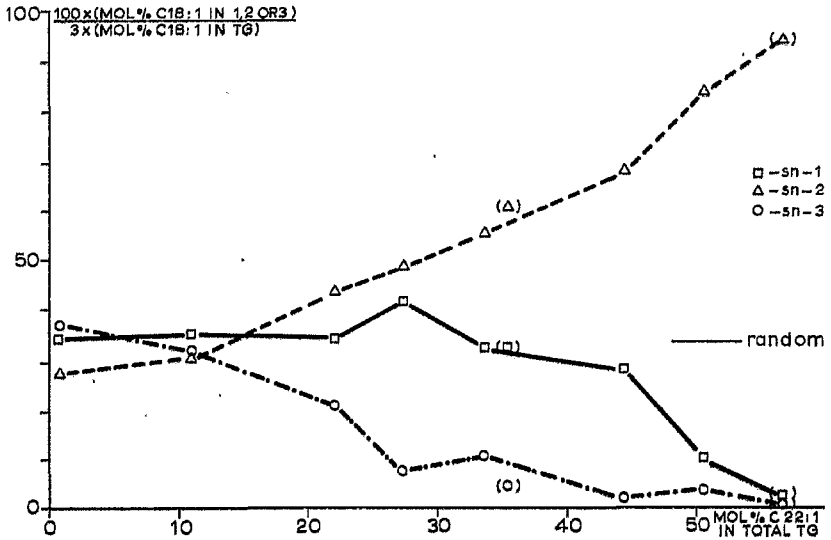


Figure 7:

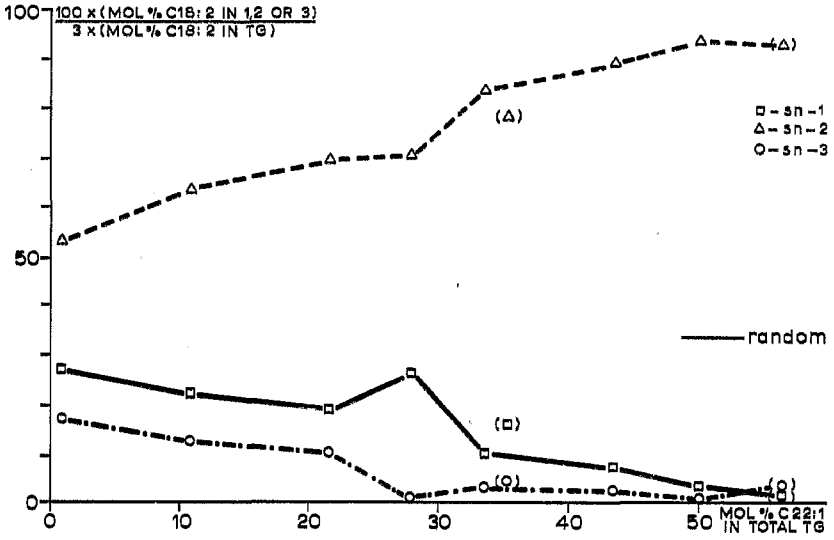


Figure 8:

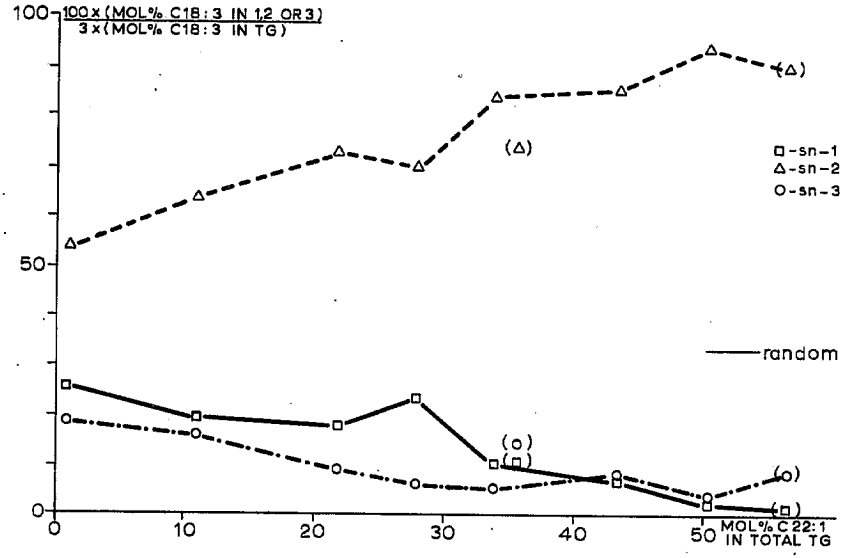
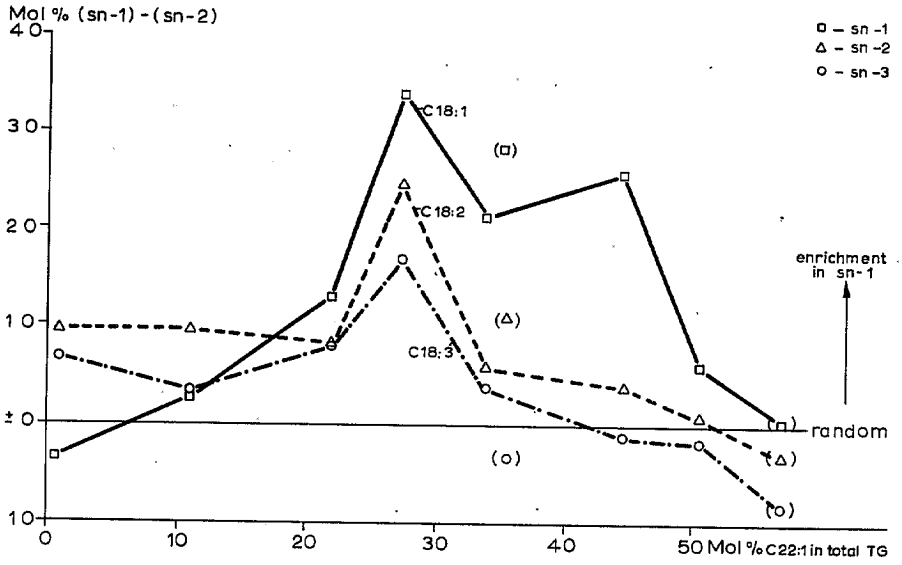


Figure 9:



The linoleic acid also shows a random distribution (Fig. 7) in species high in erucic acid, but a strong accumulation to the sn-1-position in the others.

The linolenic acid has the same over-all distribution pattern, shown in figure 8, but the asymmetry is not as pronounced as for linoleic acid.

Figure 9 gives a survey of the positional distribution of the Category II acids between sn-1 and sn-3. The curves represent the deviations from random distribution using the differences (mole per cent at sn-1 minus mole per cent at sn-3). It is clearly evident that the non-random distribution decreases with increasing unsaturation.

References

1. AKESSON, B. (1969):
Eur. J. Biochem. 9, 463

2. BROCKERHOFF, H. (1965):
J. Lip. Res. 6, 10

3. BROCKERHOFF, H. and M. J. YURKOWSKI (1966):
J. Lip. Res. 7, 62

4. BROCKERHOFF, H. (1967):
J. Lip. Res. 8, 167

5. CHRISTIE, W. W. and J. H. MOORE (1969):
Biochim. Biophys. Acta 176, 445

6. EVANS, C. D., D. G. McCONNELL, G. R. LIST and S. R. SHOLFIELD
( ):
J. Am. Oil Chem. Soc.

7. LITCHFIELD, C. (1971):
J. Am. Oil Chem. Soc. 48, 467

8. LUDDY, F. E., R. A. BARFORD, S. F. HERB, P. MAGIDMAN and
R. W. RIEMENSCHNEIDER (1964):
J. Am. Oil Chem. Soc. 41, 693

9. MATTSON, F. H. and L. W. BECK (1956):
J. Biol. Chem. 219, 735

10. MATTSON, F. H. and R. A. VOLPENHEIM (1968):
J. Lip. Res. 9, 79

11. MATTSON, F. H. and R. A. VOLPENHEIM (1968):
J. Biol. Chem. 236, 1891

12. PODLAHA, O. and B. TÖREGARD:
7. Scandinavian Symposium of Lipids, Röros, Norway