High Order Compositeness in Natural Fats. The Glyceride Structures of Seed Fats from *Schleichera trijuga*, *Sapindus trifoliatus*, and *Mimusops elengi*

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Abstract

The glyceride-type structures of seed fats from *S. trijuga* (Sapindacae, Sm 28.0), *S. trifoliatus* (Sapindacae, Sm 12.6), and *M. elengi* (Sapotacae, Sm 29.6) were studied by the gravimetric azelaoglyceride technique. The seed fats from *S. trifoliatus* and *M. elengi* agreed well with the requirements of the Glyceride-Type Distribution Rule (GTDR). The *S. trijuga* seed fat however contained GS_3 – nil, GS_2U – 25.2, GSU_2 – 34.1, and GU_3 – 40.7 percentage mols respectively against GTDR values of 0, 21.0, 42.5, and 36.5 percentage mols.

Structure deviations from GTDR of the type shown by *S. trijuga* seed fat have been designated as "HOC deviations" for obvious reasons and may probably be attributed to a new phenomenon termed high order compositeness of fats, which is ultimately caused by the nonhomogeneity of fat tissues. Since this phenomenon appears to be rather widespread, a new constant, the HOC index, is suggested to denote the degree of HOC shown by specific natural fats with reference to GTDR as the standard. The nature of further experimental evidence, required to establish the causative factors behind HOC variations, is also discussed in some detail.

Introduction

KARTHA FIRST REPORTED (1–3) that the glyceride-type structures of a large number of vegetable and animal fats, as determined by the gravimetric azelaoglyceride technique, agreed well with those calculated according to an empirical rule, GTDR. Initially (1–3) GTDR held for about 20 GS_3 – nil seed fats[1] with Sm 8–62 (saturated acids, molecules percentage) and GS_3 factor[2] 0–22 (random-random distribution), one fruitcoat fat (palm oil) of SM 54 and GS_3 factor 8, plus five animal fats, of which three were mammalian depot fats with Sm 60–68 and GS_3 factor 7–10.

GTDR was subsequently found operative for *Mangifera indica* seed fat (4) of Sm 53 and GS_3 factor 15 and for *Myristica beddomei* seed fat (5) of Sm 62 and GS_3 factor 24. A more important finding was that GTDR held for four Myristicacae seed fats with Sm 74–95 and GS_3 factor nil, namely, two *Myristica malabarica* seed fats of Sm 82 and 74 (6,7), one *Myristica attenuata* seed fat of Sm 77.5 (8), and one *Myristica fragrans* seed fat of Sm 92.6 (9). It also held for a number of miscellaneous GS_3 – nil fats with Sm below 30 (10)—50 fats in all.

As the studies were extended to seed fats from new genuses and families, a new, unexpected, and rather widespread trend in glyceride structure was gradually revealed. This particular characteristic pattern showed a deviation from GTDR requirements and has been noted in a substantial number of new fats. The first example was the seed fat of *Entada pusaetha* of the Leguminacae family. This had Sm 17.4 and contained GS_2U – 13.0, GSU_2 – 26.0, and GU_3 – 61.0 percentage mols against 8.5, 33.5, and 56.0, respectively (11). Exhaustive checking of the fat for all hitherto known types of nonfatty impurities which can interfere with azelaoglyceride analysis established that the deviation was not caused by the presence of any nonfat impurity. In GS_3 – nil fats of Sm below 30 all the current ideas of glyceride structure, such as Vander Wal's theory (12), Gunstone's theory (13), and the α-β lipase theory (14) call for the same glyceride-type structure as GTDR. Hence the deviation was outside the scope of any present structure theory.

According to the α-β lipase theory, the glyceride-type structure of any natural fat is practically the same as that required by GTDR throughout the range of natural fats thus far examined although the theoretical calculations to derive them are a good bit more involved. The configuration, however, can vary from 100% symmetry to 100% unsymmetrical types in GS_2U and the reverse in GSU_2 without causing any appreciable changes in the type structures. Hence the new trend is discussed in reference to the simple GTDR standard, particularly since it concerns only the type of structures at present.

It has been shown earlier by direct isolation and analysis (15) that fats at different locations of the same morphologic tissues can show considerable variations in component acid composition. These variations have been not only irregular but also of different patterns in different seeds and therefore did not lend themselves to statistical treatment. Yet the studies established the theory that possibly all natural fats are mixtures of component fats of differing fatty acid composition. When a composite fat is made by mixing equal proportions of two GTDR fats with different Sm, it is observed that the glyceride structure of the composite can vary from that required by GTDR for a fat of the same Sm as the composite.

Only variations produced by the admixture of GS_3 – nil GTDR fats are considered in the present communication. Two examples are given. The first concerns two such fats of Sm 10 and 20. When they are combined, the mixture will have a composition of GS_2U – 7.0, GSU_2 – 31.5, and GU_3 – 61.5 percentage mols against 6.0, 33.0, and 61.0 respectively as required by GTDR for Sm of 15.0. The compositeness cannot be established by analysis of the glyceride structure alone since the deviations from GTDR are within the limits of experimental error. This may be termed low order compositeness (LOC). Since all natural fats are composites, it is obvious that only LOC fats can show good agreement with GTDR. In-

[1] G, S, U, and A stand for glyceryl, saturated acid, unsaturated acid, and azelaic acid radicals, respectively.
[2] GS_3 random—GS_3 found has been termed the GS_3 factor.

deed, fats which have shown such agreement so far are all LOC fats.

The second example deals with two GTDR fats of Sm 10 and 50, combined in equal proportions. The mixture will contain $GS_2U - 31.0$, $GSU_2 - 28.5$, and $GU_3 - 40.5$ percentage mols against the 23.0, 44.0, and 33.0 called for by GTDR in a fat of Sm 30. The glyceride types are appreciably different from the requirements of GTDR, and the compositeness of the mixture can be established by structural analysis. This may be termed HOC. Obviously there is a quantitative relation between the proportions of glyceride types in GS_3 – nil HOC fats and their corresponding GTDR values. This is expressed in the following equation:

$$GS_2U_{comp.} - GS_2U_{GTDR} = GU_{3comp.} - GU_{3GTDR} = \tfrac{1}{2}\,(GSU_{2GTDR} - GSU_{2comp.})$$

where comp. denotes composite fat.

The deviations from GTDR, shown by *Entada pusaetha* seed fat, agreed with the requirements of the HOC equation. It was suggested that probably the deviations were caused by HOC (11). In this communication a report is given of the analysis by direct estimation methods of *S. trijuga* seed fat, which shows a marked degree of HOC, along with the seed fats of *S. trifoliatus* and *M. elengi*, which provide unusually good examples of LOC fats.

The component acids of specimens of *M. elengi* (16), *S. trijuga* (17), and *S. trifoliatus* (18) seed fats have been reported in the literature. No reports of the glyceride structure of any of these, with the use of reliable, direct estimation methods, have been recorded to date. On the basis of pancreas lipase hydrolysis results, the structure of a specimen of *S. trijuga* seed fat of Sm 27.4 has been given as $GS_3 - 0.5$, $GS_2U - 16.7$, $GSU_2 - 47.3$, and $GU_3 - 35.5$ percentage mols (19). The Sm of the present specimen happens to be practically the same as that of the sample which was studied by the pancreas lipase hydrolysis technique (19).

Experimental

The *M. elengi* and *S. trifoliatus* seed fats which were used for the experiment were prepared in the laboratory by petroleum ether extraction of the seeds. The *S. trijuga* seed fat was obtained from a reliable drug dealer, who had expressed the seeds in a country *ghani*. The relevant physical and chemical characteristics of the KOH-sulfuric ether-refined oils, required for calculating the glyceride structure, are given in Table I. The samples were unusually "clean" in that many of the nonfat impurities of various kinds which had been noted in several of the new fats under investigation were absent.

There was little unsaponifiable matter (0.5% only), no unesterifiable or esterifiable resin acids, and no lactonic material in Bertram acids. [These are recoverable from insoluble magnesium salts after the fat or the mixed fatty acid esters have been oxidized by the acetic acid-acetone permanganate procedure (22) and the hydrolyzed oxidation products have been submitted to a magnesium salt separation under specified conditions (6).]

Lactonic material in Bertram acids is estimated by lead salt crystallization of the latter from 96% ethanol when the former remains in solution as material of zero neutralization value. The higher saturation acids present will be more or less quantitatively precipitated as insoluble lead soaps. Bertram acids from nontriglyceride sources were estimated by comparing these obtained by direct oxidation of the fat with that obtained by oxidation of the pure fatty acid esters recalculated to the fat basis. None was present in any of the three fats.

TABLE I

Characteristics of Seed Fats from *M. elengi*, *S. trifoliatus*, and *S. trijuga*

	M. elengi	*S. trifoliatus*	*S. trijuga*
1. % Oil, dry seed kernel	20.0	40.0	.....
2. MpC, refined fat, closed capillary, pt. of complete fusion	9.4	6.6	26.6
3. Iodine value, Hanus', 30 min	81.0	74.0	72.0
4. Hehner value	94.4	93.8	94.7
5. % Unsaponifiables	0.4	0.5	0.5
6. Insoluble acids			
% fat	94.0	93.3	94.2
mp	45.0	36.1	40.5
iodine value	83.0	78.0	75.0
7. % Unesterifiable acids: methyl alcohol-sulfuric acid esterification	0.0	0.0	0.0
8. % Lactonic material in Bertram acids	0.0	0.0	0.0
% Bertram acids from nontriglyceride sources	0.0	0.0	0.0
9. % Mixed fatty acids in fat	94.0	93.3	94.2
% Triglycerides in fat	99.0	97.5	99.0
10. Bertram acids on fat, I	28.1	12.2	26.7
II	28.2	12.5	26.2
mean	28.1	12.4	26.5
11. Correction for lactones and Bertram acids from nontriglyceride sources	0.0	0.0	0.0
12. Saturated fatty acids			
% on fat	28.1	12.4	26.5
% in mixed fatty acids, wt	29.8	13.3	28.1
mp	61.7	60.0	59.2
mean mol wt	284.0	300.0	280.0
13. Mean mol. wt. dibasic acids assumed	188.0	188.0	188.0
14. % Saturated acids in mixed fatty acids, mols.	29.6	12.6	28.0
15. % Unsaturated acids in mixed fatty acids, mols.	70.4	87.4	72.0

For determining glyceride structure the GS_3 was obtained by the revised crystallization procedure wherein the fat was crystallized from three volumes of acetone, which contained a trace of methyl alcohol, for 72 hr at 25C. The filtered precipitate was crystallized from the same volume of acetone a second time for 24 hr at 25C (20). No precipitate was obtained in 72 hr in all three cases; hence GS_3 was absent.

The absence of GS_3 has been confirmed by an independent technique, as follows. The saturated fatty acids from the three fats, *M. elengi*, *S. trijuga*, and *S. trifoliatus* had melting points of 62.0, 59.0, and 60.0C respectively (Table I). Since the GS_3 in natural fats generally shows a melting point 2C higher than the saturated acids they are derived from (21), the GS_3 from these three fats would have melting points of 64, 61, and 62C. According to the melting-point variation rule (21), the presence of 0.2 and 0.1% GS_3 of the melting point would result in a melting point of 35.0 and 31.8C for *M. elengi* fat, 32.0 and 28.8C for *S. trijuga* fat, and 33.0 and 29.2C for *S. trifoliatus* seed fat. Against this the experimentally determined melting points for the three fats were 9.4, 26.6, and 6.6C (Table I). Hence the fats could not contain more than traces of GS_3 as shown by crystallization.

The GS_2U was estimated as GS_2A, according to the acetic acid acetone permanganate oxidation and magnesium salt azelaoglyceride separation earlier described (22). The mean molecular weight of the dibasic acids was assumed to be 188 for calculations of structure. In view of the significance of the present results in connection with the phenomenon HOC, the full details of the structural analysis are given in Table II.

TABLE II

Glyceride Structure of Seed Fats from *M. elengi*, *S. trifoliatus*, and *S. trijuga*

		M. elengi	*S. trifoliatus*	*S. trijuga*
1. GS_3 by crystallization		nil	nil	nil
2. Wt fat oxidized, g	I	1.194	1.959	1.306
	II	1.084	2.022	1.055
3. % IAG[a] on fat	I	50.1	23.9	45.6
	II	52.6	18.8	40.2
4. Iodine value of S in IAG	I	2.2	8.1	1.7
	II	2.0	5.9	2.8
5. IAG corrected for No. 4	I	49.9	23.5	45.4
	II	52.4	18.5	40.0
6. % S from SAG[b], fat basis	I	1.6	1.1	1.8
	II	0.5	3.3	3.0
7. GSA_2 corresponding to No. 6	I	4.0	2.7	4.5
	II	1.2	7.9	7.4
8. Apparent azelaoglyceride number[c]	I	53.9	26.2	49.9
	II	53.6	26.4	47.4
	mean	53.8	26.3	48.7
9. Corrections for nonfat lactones and Bertram acids from non-triglyceride sources		0.0	0.0	0.0
Azelaoglycerides on fat basis, %		53.8	26.3	48.7
Azelaoglyceride number[d]		54.4	27.0	49.2
10. % S in GS_2A-GSA_2 mixture		51.7	47.1	54.4
11. % S in pure GS_2A (S and A as in Table I)		71.5	72.6	71.3
% S in pure GSA_2 (S and A as in Table I)		40.7	42.0	40.3
12. % GS_2A on triglycerides		19.4	4.5	22.4
% GSA_2 on triglycerides		35.0	22.5	26.8
13. % GS_2U on triglycerides, wt		21.7	5.0	25.1
% GSU_2 on triglycerides, wt		44.4	28.4	34.1
% GU_3 on triglycerides, wt (by difference)		33.9	66.6	40.8
14. % GS_2U on triglyceride mols		21.6	4.8	25.2
% GSU_2 on triglyceride mols		44.4	28.0	34.1
% GU_3 on triglyceride mols		34.0	67.2	40.7

[a] IAG is material recovered from insoluble magnesium salts in azelaoglyceride separation.
[b] SAG are azelaoglycerides from soluble magnesium salts in azelaoglyceride separation.
[c] This is apparent azelaoglyceride number-saturated material in IAG plus GSA_2 in SAG on neutral fat basis.
[d] The azelaoglyceride number is GS_3 plus GS_2A plus GSA_2 on triglyceride basis.

Results and Discussion

The glyceride-type structures of the three fats, as directly estimated, are compared with GTDR values in Table III. For *S. trifoliatus* and *M. elengi* fats the differences between found and GTDR values are within 1% for GSU_2 and within 0.5% for GS_2U and GU_3. This agreement is remarkable and supports the basic significance of GTDR in the biosynthesis of natural fats. *S. trijuga* seed fat, however, showed a pronounced deviation from GTDR. GS_2U and GU_3 were 4.2% higher and GSU_2, 8.4% lower. The deviations from GTDR which were shown by *S. trijuga* seed fat agree with the HOC equation described above and probably are caused by the same HOC phenomenon as in the case of *E. pusaetha* seed fat (11).

The HOC Index

The HOC fats from different biological sources show varying degrees of compositeness, and a convenient method for indicating the degree of compositeness shown by each specimen would be helpful in developing the glyceride structure field. In view of the fact that in GS_3 – nil fats the deviations in the different glyceride types caused by HOC are simply interrelated with GTDR values, a clear idea of the degree of compositeness in such fats can be given by the deviation observed in GS_2U, GSU_2, or GU_3. However in GS_3 – nil fats the glyceride type which can be most accurately determined by direct estimation methods is GS_2U hence it is probably better to indicate the degree of compositeness by the factor $GS_2U_{comp.} - GS_2U_{GTDR}$, which may be termed the HOC index.

Since it is essential to mention whether the particular fat is of the GS_3 – nil type or not, the HOC index may be expressed thus:

$$GS_3 - \text{nil}, GS_2U = (GS_2U_{comp.} - GS_2U_{GTDR})$$

In the present examples the HOC indices for *S. trifoliatus*, *M. elengi*, and *S. trijuga* seed fats are GS_3 – nil, $GS_2U + 0.3$; GS_3 – nil, $GS_2U - 0.4$; and GS_3 – nil, $GS_2U + 4.2$ respectively.

TABLE III

Comparison of the Glyceride-Type Structures of Seed Fats from *S. trifoliatus*, *M. elengi*, and *S. trijuga* with GTDR Requirements

	S. trifoliatus	*M. elengi*	*S. trijuga*
1. Sm	12.6	29.6	28.0
2. GS_3 found	nil	nil	nil
3. GS_2U found	4.8	21.6	25.2
4. GSU_2 found	28.0	44.4	34.1
5. GU_3 found	67.2	34.0	40.7
6. GS_2U_{GTDR}	4.5	22.0	21.0
7. GSU_2 GTDR	29.0	44.0	42.5
8. GU_3 GTDR	66.5	34.0	36.5
9. GS_2U found – GS_2U GTDR	+ 0.3	– 0.4	+ 4.2
10. GSU_2 found – GSU_2 GTDR	– 1.0	+ 0.4	– 8.4
11. GU_3 found – GU_3 GTDR	+ 0.7	0.0	+ 4.2

HOC, a Characteristic of the Biological Source

Since HOC occurs only in some fats and not in others, it is in some way or other a characteristic of the biological source. In the Leguminacae family, seed fats of *Arachis hypogea* (3), *Trigonella foenugreecum* (3), *Pongamia glabra* (3), and *Adenathera pavonina* (23) are of the LOC type while *E. puaetha* seed fat has HOC index GS_3 – nil, $GS_2U + 4.5$. Similarly in Sapindacae, *S. trifoliatus* contains an LOC fat whereas *S. trijuga* has an HOC fat. Hence it is obvious that HOC is not a characteristic of any natural family as a whole. Whether it is characteristic of genuses as a whole and whether the HOC index will show variations within the same genus has yet to be discovered.

Nature of Further Experimental Evidence Required to Establish that HOC Deviations Are Caused Entirely by Nonhomogeneity of Fat Tissues

It has been established more or less conclusively that a certain amount of nonhomogeneity (NHY) of component acid composition occurs in practically all fat tissues so far examined (15), but this cannot be taken to mean that HOC deviations are caused entirely by NHY. Even if they were, the truth cannot be established by the qualitative sectioning and analysis technique (15), which is the sole experimental procedure available now. To establish the cause of HOC deviations unequivocally, it has to be shown that the magnitude of the HOC index is accounted for by the degree of NHY present. If it is not feasible to measure the degree of NHY experimentally, then the matter must be decided by other suitable means. Glyceride structure deviations from GTDR can be caused not only by NHY but also by differences in the mechanism of biosynthesis in the cells.

The type of variations discussed earlier form only part of the structure features of HOC fats. There are a number of others which can be theoretically deduced, then experimentally followed up in due course. However no feasible biosynthesis theory which explains the HOC deviations is yet available, and the detailed characteristics of fats possible according to such a theory can be worked out only after the theory itself is propounded.

Isolation and analysis of fats from individual calls may not be possible in the near future, and the NHY to be observed by sectioning and analysis would represent only the overall or minimal NHY—or to coin a more descriptive term, the physically visible NHY. This last would more or less represent true NHY only if there were complete segregation of cells of different component acid composition, in other words, only if

there exists fully segregated compositeness wherein cells of particular component acid composition are compactly clustered together to the exclusion of all others. If this does not prevail, then part of the NHY present will be physically nonvisible since it is caused by "nonsegregated" or "interdispersed" compositeness wherein cells of higher Sm (or lower) are irregularly thrown among cells of lower Sm (or higher) to produce either physically visible or physically nonvisible compositeness according to the pattern of dispersion. No direct experimental methods are available at present to ascertain whether physically nonvisible compositeness is present in fat tissues. If interdispersed compositeness prevails, then the physically visible NHY will not be able to explain the magnitude of the HOC index shown by the fat.

It was interesting to learn whether the physically visible NHY can account for the HOC index for *S. trijuga* seed fat; hence, a consignment of the seeds was obtained even though the seeds were not large in size or the HOC index high compared with many others since studied. The seed kernels, however, turned out to be unusually soft. The boundary membranes of the fat cells were easily broken, and the kernels exuded considerable oil when dissection was attempted with the sharpest edges available. If oil exudes freely during sectioning, there has been extensive rupturing of the fat cells and the seeds are considered unsuitable.

This comparative study has been conducted on other seeds, under different authorship, and it may be stated that the physically visible NHY, even when high, is not able to account for the HOC index or even a major portion of it in many cases. Hence the conclusion is that HOC is caused by physically visible NHY as well as physically nonvisible. New modes of biosynthesis also may contribute to the magnitude of the HOC index. It is likely that new procedures must be devised. A good number of HOC fats with the maximum possible HOC indices and with highly diversified component acid composition may have to be investigated in detail before the reasons for the HOC phenomenon are fully elucidated.

The phenomenon itself is of vital importance, however, since it demonstrates that the glyceride structures of all natural fats are by no means regulated entirely by any current structure theory, further that the structures and configurations of natural fats, as predicted by any present theory, may in reality be quite far removed from the true figures. This risk will be particularly high in fats from new biological sources, in which observance of GTDR has not previously been established by direct estimation techniques.

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