XIV. SOME PECULIARITIES IN THE GLYCERIDE STRUCTURE OF LAUREL FATS.

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THE broad differences between the types of synthesis that occur in three important sources of natural fats, the seed kernel, the fruit pulp and the animal tissues, were discussed in a previous communication [Collin and Hilditch, 1929], where it was suggested that, whilst the syntheses in the fruit pulp and animal tissues were very similar as far as the manner in which the available fatty acids were arranged as glycerides, the kernel synthesis evidently proceeded in a rather different way, and kernel fats were thereby endowed with a characteristic structure. It was inferred from a consideration of the association ratios (the measure of the degree of dispersion of the unsaturated acid throughout the fat) of the various kernel fats studied, that their structure depends essentially upon an unusually thorough dispersion of the fatty acids among the completed glyceride molecules. Evidence of such a structure was given by all the kernel fats examined with the exception of a sample of the commercially expressed oil of laurel (*Laurus nobilis*). Although this sample was expected to contain a certain amount of pulp fat and therefore to show some irregularity, the structure it actually disclosed was so anomalous that specimens of the true laurel kernel and laurel pulp fat from *Laurus nobilis* berries were prepared in these laboratories and analysed by similar methods for further and more reliable information.

The suggestion in the earlier paper [1929, p. 1278], that the observed abnormality might be due to admixed pulp fat, must be withdrawn for it now appears that the pulp fat is a perfectly normal specimen of its class, and that the anomaly lies entirely in the kernel fat, where the expected even mixed glyceride structure is absent and is replaced by one of extreme heterogeneity.

According to the association ratios, which are approximately constant throughout the kernel fat group, the persistent saturated-unsaturated glyceride mixture in a normal kernel fat contains about 1.4 molecules of saturated acid associated with every molecule of unsaturated acid; and, apparently, when the normal synthesis is taking place, no fully saturated glycerides are formed until this ratio is exceeded in the original mixture of acids. When the proportion of saturated acids in the mixture exceeds this ratio, formation of

saturated-unsaturated glycerides with an average association ratio of 1.4:1 continues until all the unsaturated acids are accounted for and the surplus saturated acids appear as fully saturated glycerides.

The mixture of fatty acids in the laurel kernel fat now studied had the following composition (excluding unsaponifiable): lauric 43.1 %, palmitic 6.2 %, oleic 32.3 %, linoleic 18.4 %. The molecular ratio of saturated to unsaturated acid in this mixture is very near the critical value of 1.4:1 and hence is ideally adjusted for complete conversion into mixed saturated-unsaturated glycerides with formation of no, or at the most of insignificant amounts of, fully saturated material. Actually, however, the kernel fat contains about 36 % of saturated glycerides, and here obviously the more usual mechanism of kernel fat synthesis has not been in operation. The composition of the fatty acid mixture from the saturated glycerides (lauric 95.5 %, palmitic 4.5 %) shows, moreover, that trilaurin is the predominant constituent to the extent of about 90 % of the whole; and it may be recalled that Bömer and Ebach [1928] were also able to isolate about 30 % of trilaurin from laurel oil by direct crystallisation. It should also be emphasised, in view of what follows, that although the saturated portion of the fat is so rich in lauric glycerides the remainder contains only about a quarter of the total lauric acid present in the fat.

A further difficulty is provided by the behaviour of the unsaturated acids. The limiting values for the content of tri-unsaturated glycerides can be calculated from the association ratio of the mixed saturated-unsaturated (and tri-unsaturated) glycerides and must lie between 6 and 31 %. If the heterogeneity observed in the saturated acids extended to the unsaturated acids, as was expected, then the true value would approach the higher limit. In attempting to trace such a correspondence, a part of the fat was hydrogenated as completely as possible (thus transforming all the tri-unsaturated constituents to tristearin) and fractionally crystallised from ether. Although such a method is not strictly quantitative, tristearin is so insoluble in cold ether that it may be isolated fairly completely; in this case the amount of tristearin found was small enough to justify the conclusion that the true value for the tri-unsaturated content approaches the minimum. Thus the unsaturated acids show no tendency to link up with each other after the example of the saturated acids, but distribute themselves in the characteristic way among the residual palmitic and lauric acids left over from the abnormal saturated glyceride formation.

It is difficult to reconcile the behaviour of the two groups of acids without concluding either that they were in different parts of the seed at the time of their conversion into glycerides, or that they arrived in the seed over different periods. The latter suggestion not only covers the behaviour of the acids but also offers some explanation of the peculiar glyceride structure of the fat; for, assuming that the bulk of the lauric acid appears alone in the kernel during the early stages of ripening, then trilaurin alone would be synthesised until,

as the ripening proceeded, the appearance of the unsaturated acids and the rest of the lauric acid would allow the kernel to resume the normal mixed glyceride synthesis, without however affecting the trilaurin already formed. Palmitic, the other saturated acid, would appear from the experimental data to have spread itself over the whole process, reaching its greatest concentration in the second stage of the synthesis along with the unsaturated acids.

A stepwise construction of the fat on these lines would therefore readily account for its structural divergence from the ordinary kernel fat.

EXPERIMENTAL.

The outer pulp was removed without difficulty from the laurel berries leaving a cleanly separated kernel; both pulp and kernels were steeped in acetone to dry and to remove excess fat, ground to a meal, extracted with ether in a Soxhlet and the fat combined with the acetone extract. The berries yielded 70 % kernel (fat content 14 %) and 30 % pulp (fat content 37 %); or, calculated on the original weight of berries, 10.1 % kernel fat and 11.2 % pulp fat.

The kernel fat was solid and light brown in colour. The pulp fat was a dark green liquid, which on long standing deposited small nodules of solid. They possessed the following characteristics:

	Sap. equiv.	Iodine value
Kernel fat	257.4	84.0
Pulp fat	285.7	113.1

The preliminary treatment of each fat followed the same course. They were saponified with 10 % alcoholic potash for 6 hours and the liberated acids separated into "liquid" and "solid" acids by a modification of the Twitchell process. In each case, also, before esterification the "solid" and "liquid" acids were reconverted to soap by the addition of excess of alcoholic potash and the unsaponifiable material removed by repeated extraction with ether.

	"Solid" acids g.	Non-sap. g.	"Liquid" acids g.	Non-sap. g.
Kernel fat	32.5	—	39.3	20.2
Pulp fat	26.0	0.6	55.1	7.7

The methyl esters of the purified "solid" and "liquid" acids were prepared and fractionally distilled in the usual manner, and the analytical data thus obtained are summarised below.

Laurel kernel fat.

<i>Laurel kernel fat.</i>						% of fatty acids			
						"Solid"	"Liquid"	Total	Excluding non-fatty material
						acids (35·3 %)	acids (64·7 %)		
Lauric	...	...	...	...	...	25·0	8·2	33·2	43·1
Palmitic	...	...	...	...	...	4·8	—	4·8	6·2
Oleic	...	...	...	...	...	5·5	19·4	24·9	32·3
Linoleic	...	...	...	...	...	—	14·2	14·2	18·4
Unsaponifiable	...	...	...	...	...	—	0·9	22·9	—
"	(extracted before methylation)					—	22·0		

Laurel pulp fat.

						% of fatty acids		
						"Solid" acids (29.8 %)	"Liquid" acids (70.2 %)	Excluding non-fatty material
Lauric	...	...	...	...	...	1.3	1.1	2.4
Palmitic	...	...	...	...	...	18.3	—	18.3
Oleic	...	...	...	...	...	8.0	48.9	56.9
Linoleic	...	...	...	...	...	1.5	11.1	12.6
Unsaponifiable	...	...	...	...	...	—	0.5	—
,, (extracted before methylation)						0.7	8.6	9.8

Molecular ratio of saturated to unsaturated acids 0.34:1.

Estimation of the fully saturated glycerides.

This was carried out in the usual way by treating a solution of the fat in acetone with powdered potassium permanganate and isolating the unchanged saturated glycerides from the oxidation mixture by washing with ammonia and potassium carbonate.

Laurel kernel fat.

150 g. of the kernel fat gave 42.0 g. of crude fully saturated glycerides (iodine value 1.2), and these, on further purification by boiling with aqueous potassium carbonate yielded:

- 31.9 g. (corr. wt.) neutral fat, sap. equiv. 211.1 (acid value 0.4);
- 3.2 g. (corr. wt.) fat extracted by ether, sap. equiv. 231.2 (acid value 6.2);
- 6.9 g. (corr. wt.) mainly acidic material, sap. equiv. 199.2 (acid value 45.7).

Assuming that the acidic matter present in (b) and (c) is azelaodilaurin (acid value 89.5), the proportion of fully saturated glycerides in the original material is 26.6 %; allowing for the 22 % of non-fatty compounds present in the original, the proportion of fully saturated glycerides in the true kernel fat is therefore 34.2 %.

The fully saturated portion (a) was converted into methyl esters, which gave the following results on fractionation:

g.	B.P./1 mm.	Sap. equiv.
4.28	73–93°	210.2
5.66	93	213.2
7.62	93	213.4
3.36	Residue	251.4*

* Residual esters, freed from unsaponifiable matter, sap. equiv. 228.0.

This corresponds with a composition of lauric acid 94.1 %, palmitic acid 4.3 % and unsaponifiable matter 1.6 %, or, excluding unsaponifiable matter, lauric acid 95.5 % and palmitic acid 4.5 %.

The general composition of 100 parts of the original kernel fat, based on the preceding data, is as follows:

	Total fat	Fully saturated glycerides	Mixed saturated-unsaturated or tri-unsaturated glycerides (by difference)	
	100 g.	26.6 g.	73.4 g.	
Unaponifiable	22.0	0.4	21.6	
Glycerol residue	3.9	1.5	2.4	
				Molecular ratios
Lauric	31.9	23.6	8.3	415
Palmitic	4.6	1.1	3.5	135
Oleic	23.9	—	23.9	848
Linoleic	13.7	—	13.7	489

The preponderance of lauric acid in the fully saturated portion and of palmitic acid in the rest of the fat may be noted. From the figures in the final column it appears that the "association ratio" in the mixed glycerides is about 0.4 mol. saturated acids per mol. of unsaturated acids. Consequently, of 100 parts by weight of the original kernel fat (containing 22 % non-fatty matter), 26.6 parts consist of fully saturated glycerides as described, whilst about 6–31 parts are tri-unsaturated glycerides, according as the amount of mono-unsaturated-disaturated glycerides lies between nil and 20, and that of di-unsaturated-monosaturated glycerides between 45 and nil.

Tristearin content of the hydrogenated fat. The original kernel fat (40 g.) was hydrogenated repeatedly with 5 g. nickel catalyst until the iodine value fell to 14. Great difficulty was experienced in attaining even this degree of saturation, probably because the large amount of non-fatty matter was either toxic to the catalyst or was itself difficult to hydrogenate; the residual iodine value is almost certainly to be attributed to the non-fatty constituents. The tri-unsaturated glycerides being thus converted to tristearin, the mixture (39.4 g.) was fractionally crystallised from ether. From the first crystallisation of the hydrogenated fat the following fractions were obtained by successive concentrations of the filtrate from each crop of crystals:

Crop	g.	M.P.
A	5.7	64–65°
B	4.8	49–50°
C	6.1	41–43°
D	9.4	33.5–35°
E	13.4	— (ex final mother-liquors)

On recrystallisation of crop A, a first fraction (2.8 g., M.P. 66.5–67.5°, sap. equiv. 291.2) was obtained, whilst the more soluble part melted at about 64–65° and had a sap. equiv. of 284.0. Recrystallisation of crop B yielded only a minute amount of material melting at 64–65°, the residue having a maximum melting point of about 52–53°. Since even the least-soluble and highest-melting fraction from crop A is not entirely tristearin, it is quite clear from this examination that the proportion of tri-unsaturated glycerides in the original fat cannot greatly have exceeded the minimum amount shown to be present by the previous quantitative oxidation experiments.

Laurel pulp fat.

100.5 g. of the pulp fat yielded 2.7 g. of saturated material (sap. equiv. 267.5). This corresponds with a fully saturated glyceride content of approximately 3 % on the glycerides present in the pulp fat.

The saturated compounds gave on hydrolysis fatty acids which, after removal of unsaponifiable matter, possessed an equivalent of 255.6 and a melting-point of 60–62° (unaltered on admixture with pure palmitic acid); the glyceride present was thus substantially tripalmitin.

The proportion of fully saturated glycerides, although small, is thus quite well defined, and it is interesting to observe that it is coincident with that of the corresponding synthetic triglyceride mixture as indicated by the graph correlating the proportion of fully saturated glycerides with the proportions of saturated and unsaturated acids in the mixed fatty acids of a synthetic triglyceride [Bhattacharya and Hilditch, 1930]. In common with other fruit pulp fats and with animal tissue fats, therefore, the glyceride structure of laurel pulp fat is closely similar to that of the corresponding synthetic mixed triglycerides.

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