Triglyceride Composition of Native and Rearranged Butter and Coconut Oils[1]

A. KUKSIS, M. J. McCARTHY, and J. M. R. BEVERIDGE, Department of Biochemistry, Queen's University, Kingston, Ontario

Abstract

Triglyceride gas chromatography was used for quantitative fractionation by carbon number of native and rearranged butter and coconut oils. Significant differences in the triglyceride type distributions between the corresponding native and chemically modified fats were found. Increased proportions of both short and long chain triglycerides occurred in the rearranged butterfat. In reconstituted coconut oil there was a shift towards the shorter chain length triglycerides. The natural and the rearranged triglyceride distributions of the two oils were shown to differ from the random distributions calculated for the corresponding fatty acid complements. The butterfat triglyceride compositions showed greater deviations from random than did the coconut oils. The modified oils approached random distributions more closely than did the native ones. In both cases the deviations from truly random populations appeared to be in the direction anticipated on the basis of chemical reactivity.

Introduction

IT IS GENERALLY believed that in fats composed of re-esterified fatty acids and glycerol, or in fats in which acyl migration has been promoted by a catalyst, the fatty acids are distributed in a truly random manner among the glyceride molecules (1,2). This conclusion has been arrived at despite the realization that present methods for analyzing fats in terms of their component glycerides may be too inadequate to permit complete experimental verification of the randomness of fatty acid distribution in re-esterified or rearranged fats. Although Bhattacharya and Hilditch (3) showed that the fully saturated glyceride content of such a fat conforms to the principle of random distribution, it may be questioned whether or not this demonstration is sufficient to establish the existence of a random distribution. The simple interesterification mixtures analyzed by Naudet and Desnuelle (4) also gave nearly random distributions, but certain irregularities were apparent. These observations find support in the lipase studies (5) on rearranged lard.

Pancreatic lipase hydrolysis and gas chromatographic analyses of the fatty acids of butterfat have shown (6) that the individual acyl groups are not disposed at random among all the glyceryl carbons. When considered only as saturated or unsaturated, and not as individuals, they appear to be distributed intermolecularly at random, or nearly so, but tend to assume specific positions intramolecularly. By direct triglyceride gas chromatography, it has been demonstrated (7) that the interglyceride fatty acid distribution in butter fat is also highly specific. This observation suggested the possibility that the above techniques might have failed similarly in noting a possible non-randomness in the interglyceride distribution of fatty acids in other more complex natural and rearranged or reconstituted fats. Such a belief is strengthened by the known and anticipated differences in the reactivity of fatty acids of different chain length and degree of saturation (8,9) and in the ease of esterification of the *alpha-* and *beta-*positions of the glyceride molecule (10) as well as other physical properties which are likely to have an effect on the formed structures of the natural and synthetic triglycerides. Succesful application of gas chromatography to the analysis of complex mixtures of natural triglycerides permitted a new and superior approach for evaluating the degree of fatty acid randomness in natural, rearranged, and synthetic oils. Since rearranged butterfat and reconstituted coconut oils were available it was decided to test the above hypothesis.

The following report compares the triglyceride distributions by carbon number found in native and rearranged butter and coconut oils with those anticipated on the basis of a completely random fatty acid arrangement obtained by calculation.

Experimental

All the natural and synthetic triglyceride mixtures employed in these studies were obtained from industrial companies. The native butter was obtained from Distillation Products Industries, Rochester, N.Y. A portion of this material was rearranged by Drew Chemical Corp., Boonton, N.J. The company's account of the rearrangement is given below. The butter was melted and the butter oil separated from other ingredients. It was then washed and dried, and the product reacted with 0.1% sodium methylate for approximately 1 hr at a temp of ca. 100C. The product, which became dark immediately, was washed, bleached, filtered, and deodorized. The catalyst was "killed" while the fat was still molten, precipitated as salt and filtered out. The soap content of the oil was checked and found to be nil. Some chemical and physical properties of the rearranged and the native butter are given in Table I. The native and reconstituted coconut oils were obtained from the Drew Chemical Corp. Crude coconut oil was refined, bleached, deodorized, and filtered. One portion of this

TABLE I

Some Chemical and Physical Constants of Native and Rearranged Butter and Coconut Oils[a]

Chemical or physical characteristic	Reference butter oil[b]	Rearranged butter oil[c]	Refined coconut oil[d]	Reconstituted coconut oil[e]
F.F.A. (as % oleic)	0.08	0.09	0.01	0.04
I.V. (Wijs)	34.7	31.1	10.6	10.7
Sap. value	229.8	226.1	255.1	261.5
Color (Lovibond)	70/4.9	20/3.5	4.0/0.6	30/3.2
Setting point, °C	20.2	25.7	23.5	.....
M.P. (Wiley) °F	.....	.....	77.0	86.5
Unsap. %	0.51	0.56	0.20	.....

[a] These data were supplied by Drew Chemical Corporation, Boonton, New Jersey.
[b] Blended commercial butter, a portion of which was used for rearranging.
[c] Prepared as described under Experimental.
[d] Prepared as described under Experimental.
[e] Prepared by transesterification of the fatty acid methyl esters with glycerol.

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TABLE II

Fatty Acid Composition of Native and Rearranged Butter and Coconut Oils[a]

Composition of fatty acids (wt, %)													
4:0	6:0	8:0	10:0	12:0	14:0	14:1	15:0	16:0	16:1	17:0	17:1	18:0	18:1	18:2
Native butterfat														
3.5[b]	2.3	1.1	3.0	3.0	10.0	2.0	1.4	25.5	2.0	0.5	0.5	15.2	27.5	2.5
Rearranged butterfat														
3.5[b]	2.3	1.4	3.1	3.7	12.0	2.0	(0.5)[c]	30.6	1.7	(0.5)[c]		16.0	23.7	0.0
Refined coconut oil														
	0.6	9.5	7.2	47.3	16.6			7.8				4.2	4.7	2.1
Reconstituted coconut oil														
	0.3	4.7	6.5	47.4	19.9			9.6				2.5	6.8	2.3

[a] Conditions of gas chromatography given in reference 12.
[b] Not determined; adopted on basis of work of Jensen et al. (13).
[c] Subsequent detailed analysis showed a max of 0.5% of these acids in rearranged butterfat.

oil was set aside as the original or native oil. The balance was split into glycerol and fatty acids. The total fatty acids formed were recovered as completely as possible and reconstituted into the triglycerides again in the conventional esterification technique.

The fatty acid compositions for the synthetic and the natural oils are given in Table II. The fatty acids were determined by gas chromatography as previously described (12) and the values recorded give the approximate composition by weight. The value for butyric acid was adopted from the extensive studies of Jensen et al. (13). The triglyceride gas chromatography was performed as reported earlier (11). The relative proportions of the peaks were estimated from their areas under the curves by triangulation and disc integration. A variation of up to 10% in the computed proportions of the individual peak areas was observed on repeated injection of the same sample. The random distributions and the fatty acid carbon recoveries were calculated by the methods previously described (7). The contributions of the odd carbon number triglycerides to the calculated distributions were smaller than 5% of the adjacent major peaks and were either ignored or added to the major neighbouring peaks.

Results and Discussion

Butterfat Triglycerides. Application of gas chromatographic techniques to the analysis of butterfat and its molecular distillates gave evidence for a nonrandom fatty acid distribution (7). As a further check on the soundness of this conclusion, it was of interest to determine the triglyceride distribution for rearranged butterfat. Figure 1 compares representative elution patterns for native and catalytically rearranged butterfat triglyceride samples. Although there were differences in the fatty acid composition (Table II) of these two samples, they cannot account fully for the discrepancies in the elution curves. Table III gives numerical evaluation of the molar triglyceride composition of these butterfats and compares them to their corresponding random distributions. For ease of calculation, only the even carbon number triglycerides have been considered and the entire area recorded attributed to them. The error introduced in doing this is small and has been neglected. As a result, the triglyceride distribution computed for the native butterfat resembles closely that obtained when all triglyceride peaks are considered (7). The triglyceride distribution determined for the rearranged butterfat differs greatly from that of the original fat. The characteristic pattern has been changed and the rearranged product has much more of both the shorter and the longer chain triglycerides. Studies with a variety of butterfats have failed to uncover similar triglyceride populations of native butters. A comparable glyceride distribution is obtained, however, by calculation on the basis of a random butterfat fatty acid distribution. This finding might have been expected as it has been repeatedly stressed (1,2) that catalytic triglyceride rearrangement brings about randomization of the fatty acids.

Figure 2 is based on the values given in Table III and compares graphically the random and the experimental distributions for the two butterfats. Close examination of this figure reveals the superficiality of the resemblance between the random and the experimental distributions of the rearranged butterfat triglycerides. The experimental distribution contains considerably more of C_{50}, C_{52}, and C_{54} triglycerides. Since these are the glycerides that may have been

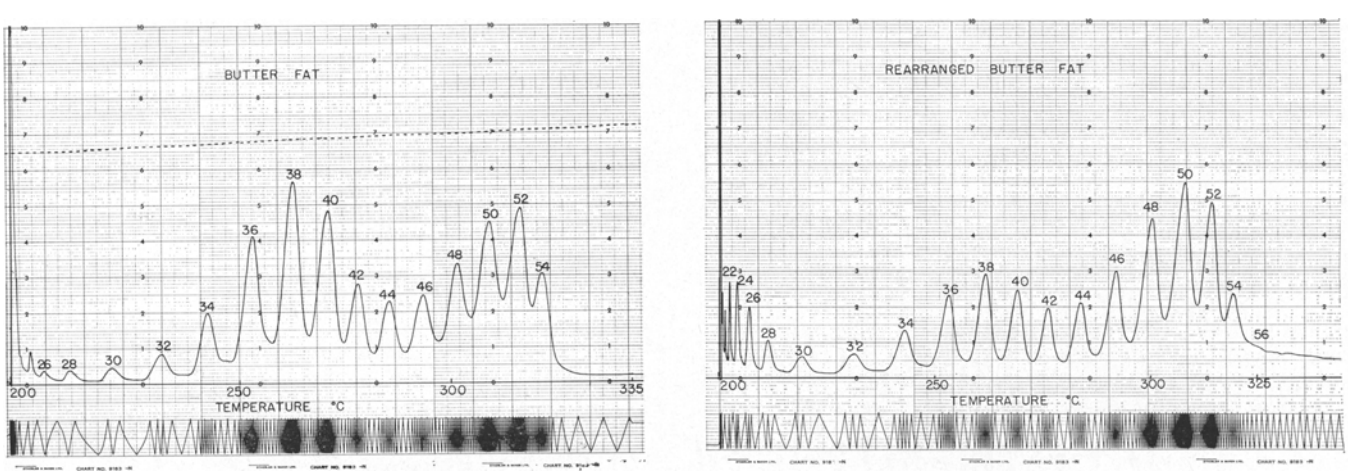

FIG. 1. GLC elution patterns recorded for native and rearranged butterfats. Chromatography conditions and carbon number assignments as previously described (11).

TABLE III

Distribution of Various Triglyceride Types in Native and Rearranged Butterfats

Triglyceride type	Distribution of triglyceride types (Moles, %)			
	Native butterfat		Rearranged butterfat	
	Experimental[a]	Random[b]	Experimental[c]	Random
C_{56}		0.5	0.1	
C_{54}	5.1	7.1	5.3	3.3
C_{52}	8.4	13.1	12.7	9.0
C_{50}	10.0	13.3	15.7	12.5
C_{48}	7.4	10.1	13.0	11.7
C_{46}	6.4	8.0	7.9	9.2
C_{44}	5.7	6.4	4.5	7.1
C_{42}	7.1	6.5	4.1	6.5
C_{40}	11.9	9.1	6.3	8.5
C_{38}	14.0	8.2	8.2	9.2
C_{36}	11.6	5.3	6.9	7.2
C_{34}	5.8	3.2	3.3	4.4
C_{32}	3.3	2.3	2.4	2.9
C_{30}	1.3	1.7	1.7	2.1
C_{28}	1.0	1.7	1.9	1.9
C_{26}	0.7	1.6	2.0	2.0
C_{24}	0.5	0.8	2.1	1.3
C_{22}	0.0	0.3	1.4	0.6
C_{20}	0.0	0.2	0.3	0.3
C_{18}	0.0	0.5	0.2	0.2
C_{16}		0.1		0.1
C_{14}		0.1		0.1
C_{12}		0.1		0.1

[a] Total fatty acid carbon recovery was 97.8%.
[b] Values adapted from reference 7, Table III, by adding to each carbon number triglyceride peak the value calculated for the preceding odd carbon number triglyceride peak.
[c] Total fatty acid carbon recovery was 102.3%.

underestimated to a maximum of 10% (7), it may be speculated that the true differences in the concentrations of the above types in the two butterfats are even greater. Similarly, should there be an error in the experimental estimation of the medium chain length triglycerides, it should appear as an overestimation. Hence the differences between the estimated and calculated proportions of the C_{46}, C_{44}, C_{42}, C_{40}, and C_{38} triglycerides may have been underestimated. The calculated and the estimated proportions of the short chain triglycerides are very close. The nearly quantitative fatty acid carbon recoveries indicate that the proportions determined for the various triglyceride types are essentially correct.

On the basis of these data, it would appear that certain non-statistical directive influences must have been operative during the rearrangement, provided the time of reaction (1 hr, 100C) was sufficient for complete equilibration and randomization. Such directive physical factors as preferential crystallization and/or dissolution would appear to be excluded on account of complete solubilization of all triglycerides at the reaction temperature. The loss in the medium chain length triglycerides and an increase in both short and long chain triglycerides in the rearranged fat point to a preferential reesterification of the short and long chain fatty acids. These relationships also indicate that the reaction has already proceeded beyond the random concentrations. For example, in the native fat the long chain triglyceride concentrations are less than those predicted for random distribution whereas in the rearranged product they are greater. Therefore, if there have been any directive influences during the process of interesterification, they must have been of a chemical nature. Slight differences in the reactivity of the individual fatty acids (8,9), coupled with the recently recognized differential esterifiability of the *alpha-* and *beta*-hydroxyl groups of the glycerol molecule (10), may have been sufficient to bring about a non-random distribution. These differences in the reaction rates would be operating during both making and breaking of ester bonds, resulting in a thermodynamic equilibrium condition which is not identical to a statistically random glyceride mixture. It seems reasonable to assume also that the fatty acids occupying the *beta*-position of a glyceride molecule would not be as readily equilibrated with the rest of the molecules as the residues on the *alpha*-position, since the former would have to migrate first to the *alpha*-position, as was shown for partial glycerides (9), before leaving their parent glycerol molecule. Such effects may be of minor importance in practical esterification, and may have remained undetected by the techniques previously used to study them.

TABLE IV

Distribution of Various Triglyceride Types in Refined and Reconstituted Coconut Oils

Triglyceride type	Distribution of triglyceride types (Moles, %)			
	Refined coconut oil		Reconstituted coconut oil	
	Experimental[a]	Random	Experimental[b]	Random
C_{54}	0.2	0.1	0.0	0.1
C_{52}	0.9	0.1	0.0	0.2
C_{50}	1.0	0.4	0.3	0.6
C_{48}	1.7	1.5	1.3	2.0
C_{46}	2.1	2.4	2.4	3.5
C_{44}	3.7	5.2	5.4	7.1
C_{42}	7.0	10.1	10.9	12.8
C_{40}	10.0	11.3	13.2	14.6
C_{38}	17.4	16.8	18.2	19.1
C_{36}	20.8	19.1	20.1	19.7
C_{34}	17.9	13.1	11.2	10.6
C_{32}	13.2	11.9	9.4	6.9
C_{30}	3.1	4.3	3.3	2.0
C_{28}	0.6	3.0	2.2	0.9
C_{26}	0.1	0.5	1.3	0.1
C_{24}	0.2	0.3	0.4	0.0
C_{22}	0.4		0.4	

[a] Total fatty acid carbon recovery 99.9%.
[b] Total fatty acid carbon recovery 97.1%.

Coconut Oil Triglycerides. Figure 3 represents the gas chromatographic runs with refined and with reconstituted coconut oils. The molar concentrations of the corresponding triglyceride types are compared in Table IV. Part of the difference in the two triglyceride populations may be attributable to differ-

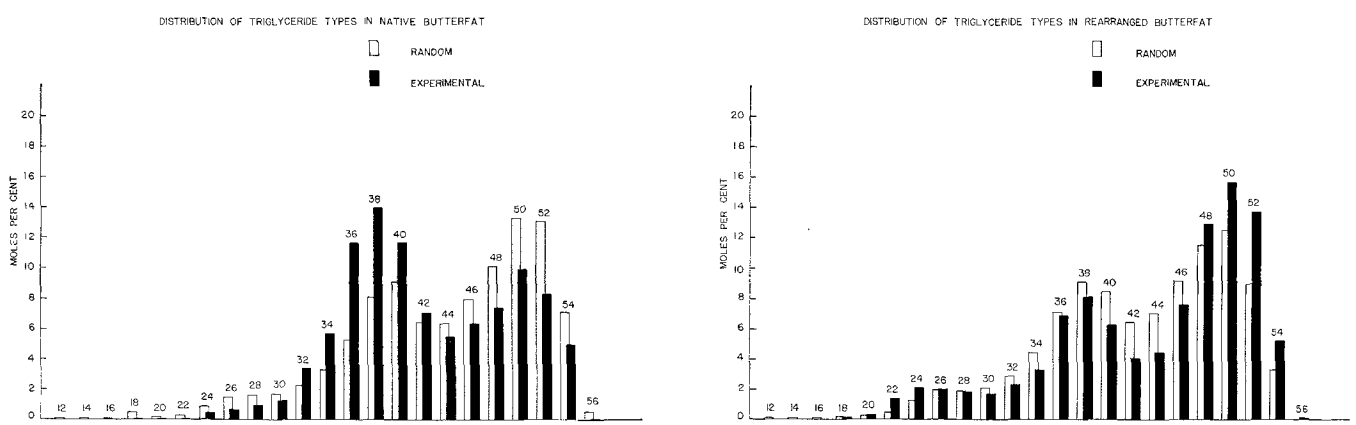

FIG. 2. Distribution of triglyceride types in native and rearranged butterfat.

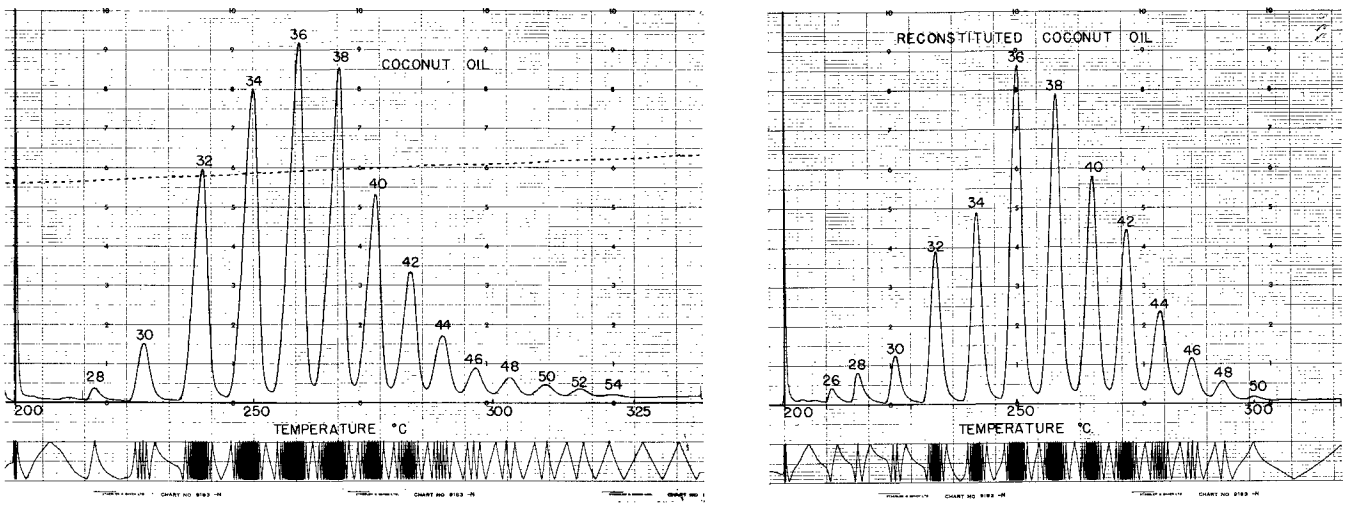

FIG. 3. Gas-liquid chromatographic elution patterns recorded for refined and reconstituted coconut oils. Chromatography conditions and carbon number assignments as previously described (11).

ences in fatty acid composition (Table II), as there had been some losses of fatty acids during their recovery from the saponified coconut oil. Among other things, one may note in Table IV that both distributions have their maxima at C_{36} and that the reconstituted coconut oil contains hardly any C_{52} and C_{54} triglycerides despite similar concentrations of long chain fatty acids in both oils, though the relative concentrations of the C_{50}, C_{48}, and C_{46} triglycerides are about the same. The reconstituted oil has more of C_{26} and C_{28} triglycerides. The considerable losses of caprylic acid during the fatty acid recovery (Table II) are reflected mainly in the decreased concentrations of C_{32} and C_{34} triglycerides, to the formation of which caprylic acid contributes most.

Table IV gives also the random distributions for the refined and the reconstituted coconut oils. The numerical values are graphically displayed in Figure 4. It may be seen from the graphs that there is a fairly close agreement between the random and the experimental distributions for both the refined and the reconstituted oils. However, there are some differences. Thus, the refined cocount oil contained considerably more of the C_{54}, C_{52}, and C_{34}, and less of the C_{28} and C_{42} triglycerides than were predicted on the basis of random distribution. The overall agreement between the experimental and the random distributions is particularly close for the reconstituted coconut oil. Even the shortage of the caprylic acid apparent in the C_{32} and C_{34} peaks is faithfully reproduced. Minor differences, which cannot be attributed simply to inaccuracies in the fatty acid and/or triglyceride estimates, are still present and must reflect true deviations from a completely random distribution. This belief is supported by the results of enzymatic positional analysis on both natural and reconstituted coconut oils (14) since both oils showed non-randomness in their fatty acid placement.

It is obvious that the reconstituted coconut oil more closely approaches the random distribution than the rearranged butterfat. Whether or not an explanation for this difference between the two oils rests in the higher oleic acid content of butterfat may be open to speculation. This unsaturated acid is known to have a much lower esterification velocity constant than the saturated fatty acids of corresponding or shorter chain length (8), at least with acid catalysts. Since in coconut oil, oleic and linoleic acids are present only in small amounts, they probably cannot greatly alter the overall random placement of the major fatty acids, lauric and myristic, both of which would be expected to have about the same chemical reactivity. Although differences in the reactivity alone of fatty acids or the positions of glycerol may throw no light on the subject when all positions of glycerol are supposed to be full, from a theoretical point of view the heat of formation of the ester bond between a fatty acid and glycerol may well be altered by the fatty acids which are on the other positions of the same glycerol molecules. If this were so, the statistically random condition and the equilibrium condition might not coincide. Aside from these considerations, for

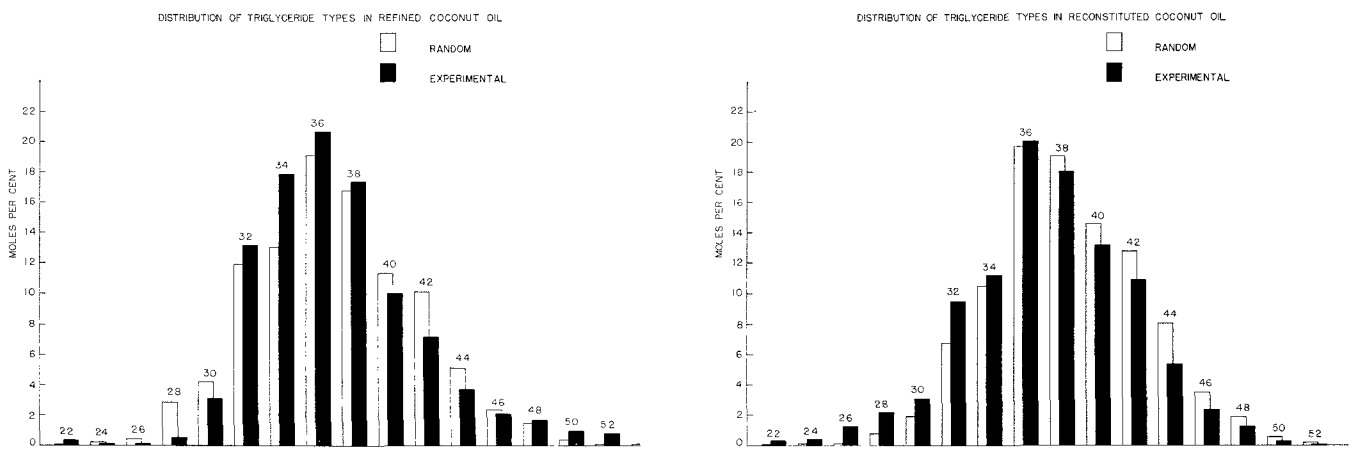

FIG. 4. Distribution of various triglyceride types in refined and reconstituted coconut oils.

the equilibrium condition to coincide with the statistically random condition, it must be assumed that the heats and entropies of mixing are zero, which appears unlikely.

Furthermore, there does not appear to be any real reason why the process of reconstitution (as in coconut oil) should result in a more random placement of the fatty acids than the process of catalytic rearrangement (as in butterfat), as the former is unlikely to be limited to esterifications involving only simple fatty acid esters and glycerol since intra- and inter-glyceride trans-esterifications should also be taking place. Therefore, both processes should lead to essentially the same end products as a result of the same directive influences. It is difficult to imagine that any interesterification reaction with such diverse mixtures of fatty acids could ever be carried out free of any selective physico-chemical influences, which would be an obligatory condition for insuring randomness.

Like the studies on the native and rearranged butterfats, the work with the coconut oils was limited to the available materials. Despite the lack of comprehensiveness it has revealed hitherto unavailable information. These results indicate the need for more exhaustive and systematic analysis of interesterified fat mixtures before the glyceride arrangements produced by the common present-day industrial processes may be pronounced as truly random.

ACKNOWLEDGMENTS

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Long Chain *a*-Phosphono Fatty Acids, Salts and Esters[1]

E. W. MAURER, A. J. STIRTON, W. C. AULT, and J. K. WEIL, Eastern Regional Research Laboratory,[2] Philadelphia, Pennsylvania

Abstract

A series of *a*-phosphono fatty acids and their salts and esters was prepared from pelargonic, capric, lauric, myristic, palmitic, and stearic acids. In comparison to corresponding *a*-sulfo fatty acids the *a*-phosphono fatty acids are white solids of higher melting point, weaker acids, less hygroscopic, have a lower critical micelle concentration and are less resistant to hard water. Methyl, isopropyl, and amyl esters RCH [PO $(OH)_2$] CO_2R', were prepared from the *a*-phosphono fatty acids; a sulfuric acid catalyst was required in the case of lower boiling alcohols. Hydrolysis studies with sodium methyl *a*-phosphonomyristate showed the *a*-phosphono ester to be 50 times as stable towards alkali as the corresponding *a*-sulfo ester, but only one-tenth as stable toward acid hydrolysis. Wetting, foaming, detergent, and other surface active properties of the *a*-phosphono fatty acids, salts, and esters were measured and compared with those of analogous *a*-sulfo compounds.

Introduction

THE SYNTHESIS and properties of *a*-sulfo fatty acids and their derivatives has been reported by this laboratory (6). It is of interest to determine the effect of an *a*-phosphono group in place of an *a*-sulfo group in the fatty acid chain. Related work by others has concerned mainly the synthesis of trialkyl esters of *a*-phosphono fatty acids and their properties as plasticizers and lubricants (1,9–12). This paper describes the preparation of *a*-phosphono fatty acids and their salts and esters from pelargonic, capric, lauric, myristic, palmitic, and stearic acids in the reaction sequence shown in Figure 1.

Methyl, isopropyl, and amyl esters, RCH [PO $(OH)_2$] CO_2R', containing 14–19 carbon atoms, were prepared from the *a*-phosphono fatty acids. *a*-Phosphono fatty acids are weaker acids than the *a*-sulfo fatty acids and it was necessary to add a sulfuric acid catalyst in the case of the lower boiling alcohols. The acids, esters, and their salts were examined for solubility, critical micelle concentration, surface and interfacial tension, Ca^{++} stability, wetting properties, detergency, foam height, and lime soap dispersing properties. Hydrolysis rate constants were also determined for the *a*-phosphono fatty acid esters.

Experimental

a-Phosphono Fatty Acids. The synthesis of *a*-phosphonopalmitic acid illustrates the general procedure.

Palmitic acid was *a*-brominated in the usual manner by the Hell-Volhard-Zelinskii reaction, followed by reaction of *a*-bromopalmitoyl bromide with absolute ethanol. Ethyl *a*-diethylphosphonopalmitate was prepared as described by Ackerman et al. (1) by heating

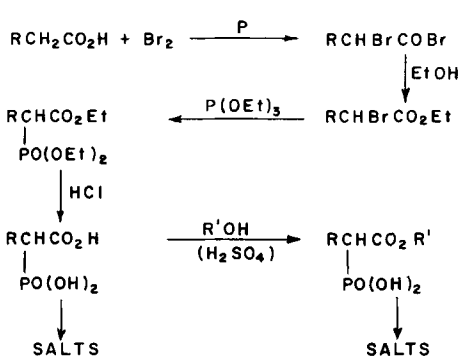

FIG. 1.

[1] Presented at the AOCS meeting, Atlanta, 1963.
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