

458. The Configuration of Naturally Occurring Mixed Glycerides. Part V. The Configuration of the Major Component Glycerides of Cacao Butter.

By M. L. MEARA.

Cacao butter has been resolved into eleven fractions by exhaustive crystallisation. From a knowledge of the component fatty acids of each fraction the component glycerides are deduced, and from these and from the data obtained on crystallising hydrogenated portions of selected fractions the presence of β -oleodistearin, β -palmito-oleostearin, β -oleodipalmitin, and β -palmitodiolein have been unequivocally proved. It is possible that α -oleodipalmitin may also be present in small amounts.

CACAO butter, although it melts below 35°, is particularly hard and brittle and softens over a narrow temperature range. It is therefore not surprising that this is a commercially valuable confectionery fat, and has been the subject of a number of investigations. Klimont (*Z. Unters. Nahr. Genussm.*, 1906, 12, 359) isolated an oleodistearin, an oleodipalmitin, and an oleopalmitostearin from this fat, and showed that simple triglycerides were absent. Amberger and Bauch (*ibid.*, 1924, 48, 371) fractionally crystallised a hydrogenated cacao butter, the data obtained, as subsequently interpreted by Lewkowitsch (*J. Soc. Chem. Ind.*, 1933, 52, 236r), indicating the presence of 56% of α -palmito-oleostearin, 26.5% of β -oleodistearin and 17.5% of β -palmitodiolein. A more comprehensive study was carried out by Hilditch and Stainsby (*J. Soc. Chem. Ind.*, 1936, 35, 95r) who separated cacao butter by crystallisation into three fractions. The component fatty acids of each fraction were determined, and from these the component glycerides were deduced to be oleopalmitostearins 52, oleodistearins 19, steardioleins 12, palmitodioleins 9, oleodipalmitins 6, palmitostearins 2% (wt.). Further they were able to isolate large amounts of β -palmitodistearin from completely hydrogenated and partly hydrogenated specimens of cacao butter. They concluded that oleopalmitostearin occurred primarily as the β -palmito-isomer, and put forward a certain amount of evidence to show that β -oleodipalmitin and β -oleodistearin were present, and also suggested the possible occurrence of both α - and β -steardiolein in cacao butter.

The present paper constitutes an attempt to isolate individual component glycerides in a state of sufficient purity for them to be examined by the methods developed in Parts I—IV of this series (*J.*, 1945, 22, 23; 1947, 773; 1948, 722).

EXPERIMENTAL.

Neutralised cacao butter (ca. 600 g.; saponification equiv., 290.0; I.V., 36.7; component fatty acids, mol.-%: palmitic 26.2, stearic 34.4, oleic 37.3, and linoleic 2.1) was exhaustively crystallised from acetone and ether at various dilutions and temperatures in a manner somewhat similar to that described in Part IV of this series. As was expected the crystallisation required was more exhaustive than that necessary for piqui-a fruit coat fat (Part III) and palm oil (Part IV) since the larger number of component glycerides renders the separations obtained on crystallisation much less complete, apparently in consequence of mutual solubility effects. Such effects have also recently been found to be acute in the case of Dika fat (component fatty acids: decoic 2.0, lauric 46.6, myristic 44.1, palmitic 5.2 oleic 1.9, linoleic 0.2 mol.-%).

The fat was finally resolved into eleven fractions (see Tables I and II). The component fatty acids of each fraction were determined by ester fractionation after a preliminary lead salt separation, and the component glycerides computed from the data obtained.

TABLE I.
Fractions obtained on Crystallisation of Cacao Butter.

Fraction.	A.	B.	C.	D.	E.	F.	G.	H.*	I.	J.*	K.
Wt. (g.)	55.3	37.1	56.5	100.5	104.5	67.8	15.9	22.4	45.6	7.9	12.3
Sap. Equiv. ...	297.9	296.4	291.3	287.9	285.1	288.7	286.9	290.9	290.9	290.9	299.3
I.V.	27.4	26.8	28.9	28.8	29.5	34.1	46.3	52.4	56.2	59.0	79.8
% (wt.)	10.5	7.1	10.7	19.1	19.9	12.9	3.6	4.3	8.7	1.5	2.3
% (mol.)	10.2	6.9	10.7	19.3	20.2	13.0	3.1	4.2	8.6	1.5	2.3

TABLE II.

Component Acids and Glycerides of Cacao Butter Fractions.

Fraction.	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.
Acid, mol.-% :											
palmitic	3.5	8.1	21.5	31.9	39.2	41.3	31.1	23.6	23.6	23.6	8.4
stearic	64.4	60.6	45.6	35.9	28.5	26.0	21.9	17.8	17.8	17.8	8.9
oleic	32.1	31.3	32.9	31.8	31.5	26.6	41.5	53.1	53.1	41.5	71.5
linoleic	—	—	—	0.4	0.8	6.1	5.5	5.5	5.5	5.5	11.2
Component glyceride groups, mol.-% :											
trisaturated	3.7	6.3	1.3	3.4	3.1	2.9	—	—	—	—	—
disatd. mono-un-											
satd.	96.3	93.7	98.7	96.6	97.9	97.1	59.0	24.2	24.2	24.2	51.9
mono-satd. di-											
unsatd.	—	—	—	—	—	—	41.0	75.8	75.8	75.8	48.1
Component glycerides (increments, mol.-%) :											
fully saturated ...	0.4	0.4	0.1	0.7	0.6	0.4	—	—	—	—	2.6
oleopalmitostearin	0.8	1.2	6.8	17.5	16.4	9.7	1.6	0.9	1.8	0.3	— 57.0
oleodistearin	9.0	5.3	3.8	1.1	3.2	—	—	—	—	—	— 22.4
oleodipalmitin ...	—	—	—	—	—	2.9	0.3	0.1	0.3	0.1	— 3.7
palmitodiolein ...	—	—	—	—	—	—	0.7	1.8	3.7	0.6	0.6 7.4
stearodiolein	—	—	—	—	—	—	0.5	1.4	2.8	0.5	0.6 5.8
linoleodiolein	—	—	—	—	—	—	—	—	—	—	0.8 0.8
triolein	—	—	—	—	—	—	—	—	—	—	0.3 0.3

* The small fractions H and J are assumed to have the same composition as fraction I.

A portion of each fraction (other than the very small groups, G, H, J, and K) was hydrogenated at 100° in the presence of Raney nickel, after which the completely hydrogenated glycerides obtained were exhaustively crystallised from ether, following the procedures developed in Part IV (*loc. cit.*).

DISCUSSION.

In this investigation it was not possible, as in earlier studies, to separate fully saturated from mixed saturated-unsaturated glycerides. Consequently the accuracy of the computation of the fully saturated components depends in some instances upon any limitation in accuracy, resulting from analysis of relatively small amounts of each fraction, since an error of, *e.g.*, 1% in the component fatty acids may give rise to, *e.g.*, 3% error in terms of component glycerides. It is possible that analyses of small quantities of material tend to underestimate slightly the content of unsaturated acids.

Fraction A, which was obtained in well-defined crystalline form, consists almost entirely of an oleo-distearin which is capable of existence in polymorphic forms melting at 23—24°, 29—30°, 36.5—37°, 43.3—43.5°, respectively, indicating the presence of β -oleodistearin. Crystallisation of the hydrogenated material gave a small fraction AH3 consisting of pure β -palmito-distearin, indicating the presence of a small proportion of β -palmito-oleostearin in fraction A. From Tables II and III it is deduced that fractions B and C were not composed of single individual glycerides but contained oleodistearin in decreasing, and oleopalmitostearin in increasing, amount. The configuration of the oleodistearin in these fractions cannot be stated categorically but it is reasonable to suppose that, since it occurred exclusively as the β -oleo-isomer in fraction A, much if not all of that in fractions B and C can be expected to have had this configuration. The oleopalmitostearin in these fractions occurred exclusively as the β -palmito-isomer since only the symmetrical palmito-distearin was observed in the respective hydrogenated palmito-glyceride fractions. Fraction D consisted of a fairly pure specimen of oleopalmitostearin which on hydrogenation gave rise to β -palmitodistearin in amounts indicative again of the virtual absence of any of the α -isomer. Similarly, β -palmitodistearin was identified in quantity in the hydrogenated fraction E together with an indication of β -stearodipalmitin originating from β -oleodipalmitin which occurred to the extent of about 16% in this fraction. The possibility of the occurrence of α -stearodipalmitin in this fraction cannot be overlooked, but, since the m. p. of EH5, the fraction of lowest m. p., ranged from 63° to 66°, large amounts of this isomer cannot have been present, otherwise the m. p. would have been considerably depressed. In the same way fraction F is very similar to fraction E in that no difficulty was experienced in obtaining relatively large amounts of β -palmitodistearin (derived from β -palmito-oleostearin), and there was evidence of β -stearodipalmitin together with, possibly, small quantities of α -stearodipalmitin. Finally fraction IH1 clearly indicates the presence of

TABLE III.

Transition and Melting Points of Fractions obtained by Fractional Crystallisation of Hydrogenated Fractions.

	G.	% of fraction.	Polymorphic forms.				M. p.*
			IV.	III.	II.	I.	
AH1	10.70	89.0	54.0	64—65	—	71.5—71.7	—
AH2	0.78	6.5	53.5—54	64—65	—	71.2—71.5	—
AH3	0.28	2.3	49—50	54—55	63—64	67.5—68	—
BH1	8.28	57.2	54.5	64—65	—	71—72	—
BH2	2.25	15.5	—	—	—	—	67—69°
BH3	1.70	11.7	49—50	54—55	63—64	67—68	—
BH4	0.53	3.7	—	—	—	—	65—67
CH1	4.89	27.9	53—54	63—65	—	70—71	—
CH2	4.68	26.7	—	—	—	—	67—68.5
CH3	4.53	26.3	49—50	54—55	63—64	67—67.5	—
CH4	0.80	4.6	—	—	—	—	65.5—67.5
CH5	0.78	4.4	—	—	—	—	64—67
CH6	0.95	5.4	—	—	—	—	64—66
DH1	6.94	36.0	50	55—56	63.5—64	67—67.8	—
DH2	2.01	10.4	49—50	55—56	63—64	67—67.5	—
DH3	3.68	19.1	49—50	54—55	63—64	66.5—67.5	—
DH4	1.44	7.5	—	—	—	—	66—67.5
DH5	2.61	13.6	—	—	—	—	64—66.5
EH1	4.81	25.4	49—50	55—56	63.5—64	67—68	—
EH2	4.18	22.2	49—50	54—55	63—64	66—67.5	—
EH3	1.95	10.3	48—49	54—55	63—64	66—67	—
EH4	3.67	19.4	—	—	—	—	64—66
EH5	1.90	10.0	—	—	—	—	63—66
FH1	4.92	33.4	49—50	55—56	64	67.5—68	—
FH2	2.91	19.7	49—50	55—56	63.5—64	67—68	—
FH3	0.97	6.6	49—50	54—55	63—64	67—67.5	—
FH4	0.88	5.9	—	—	—	—	66—67
FH5	0.80	5.4	—	—	—	—	65—66.5
FH6	0.76	5.2	—	—	—	—	64—66
IH1	3.70	25.5	54.0	64—65	—	71.5—72	—
IH2	2.07	14.3	—	—	—	—	67—69
IH3	2.55	17.6	49—50	55—56	63.5—64	67—68	—
IH4	0.95	6.5	49—50	54—55	63—64	66.5—67.5	—
IH5	0.55	3.8	—	—	—	—	64—66
β -Stearodipalmitin †			49	59	65	68	—
α -Stearodipalmitin ††			46.5	55	59.5	62.5	—
β -Palmitodistearin †			50	56	64	68	—
α -Palmitodistearin ††			50	57	61	65	—
Tristearin †			54.5	65	—	71.5	—

* Detailed thermal data were not determined for fractions which were binary mixtures, the melting points only being recorded.

† Clarkson and Malkin, *J.*, 1934, 666.

‡ Malkin and Meara, *J.*, 1939, 103.

†† Carter and Malkin, *J.*, 1939, 577.

tristearin derived from stearodiolein. The bulk of the fraction IH2, IH3, IH4 consisted of β -palmitodistearin derived from β -palmito-oleostearin and β -palmitodiolein respectively, indicating that in both of these glycerides the palmitic acid is attached to the β -hydroxyl group of the glycerol. Fraction IH5 which consisted primarily of stearodipalmitin showed evidence of the presence of the β -isomer, but the absence of the α -isomer was not precluded.

It is clear therefore that in cacao butter, a seed fat as distinct from a fruit coat fat, there is but little evidence of the presence of unsymmetrical glycerides, the following glycerides having been unequivocally identified: β -oleodistearin, β -palmito-oleostearin, β -oleodipalmitin, and β -palmitodiolein. The presence of small amounts of α -oleodipalmitin is however not excluded.

In Part IV of this series it was shown that the estimate of the component glyceride composition obtained from exhaustive-crystallisation data was in good agreement with the values obtained by applying the appropriate method of computation.

In the present investigation it is possible to compare the values obtained for the constituent mixed triglycerides of cacao butter with those determined earlier by Hilditch and Stainsby (*loc. cit.*) and with the values computed on the basis of simple "even" distribution (cf. Hilditch and Meara, *J. Soc. Chem. Ind.*, 1942, **61**, 117), a modified "even" distribution, or "random" distribution of the fatty acids amongst the glycerol molecules.

TABLE IV.
Observed and Computed Composition of Cacao Butter.

	Found.		Computed.		
	Present investigation.	Hilditch & Stainsby.	"Even."	Modified "even."	"Random."
Fully saturated glycerides	2.6	2.5	—	—	24.0
Oleodipalmitin	3.7	6.5	—	9.6	8.4
Oleopalmitostearin	57.0	51.9	75.9	57.6	21.6
Oleodistearin	22.2	18.4	11.0	20.2	13.7
Palmitodiolein	7.4	8.4	5.8	5.8	11.3
Stearodiolein	5.8	12.0	7.3	7.3	15.8
Triolein	1.1	—	—	—	5.2

The two experimentally determined sets of values agree amongst themselves. Simple "even" distribution considerably over-estimates oleopalmitostearin, primarily at the expense of other mono-oleo-glycerides. "Random" distribution grossly over-estimates the fully saturated glyceride content and to some extent the di- and tri-oleins at the expense primarily of oleopalmitostearin and to a lesser extent oleodistearin.

If, however, after partitioning oleic acid between palmitic and stearic acids the assumption (first suggested by Hilditch and Stainsby, *loc. cit.*) is made that in the mono-oleo-portions only two-thirds of the oleic acid is associated with palmitic and stearic acids as oleopalmitostearin, the remainder occurring as oleodipalmitin and oleodistearin respectively, values are obtained which are in good agreement with the experimentally determined values.

Borneo tallow, another fat in which simple computation appears to give rise to an over-estimated amount of oleopalmitostearin, is at present under similar investigation.

The author thanks Professor T. P. Hilditch for his continued advice and criticism during this investigation.

THE UNIVERSITY, LIVERPOOL.

[Received, April 13th, 1949.]