288. *The Structure of the Major Component Glyceride of Cocoa Butter, and of the Major Oleodisaturated Glyceride of Lard.*

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It was recently suggested that the major glyceride of cocoa butter, long known to be one of the three possible oleopalmitostearins, is the 2-palmitate. From cooling curves and *X*-ray and infrared analyses, it is now concluded that the glyceride is the 2-oleate. Similar observations on the disaturated glycerides of lard show, in agreement with other workers, that the major disaturated glyceride is 2-palmito-oleostearin.

Cocoa butter is known to contain a high proportion of a single glyceride. Probably as a consequence of this, it exhibits marked polymorphism, and melts over a comparatively narrow temperature range. It is brittle at room temperature and completely molten at body temperature. For this and other reasons, it has found considerable application in confectionery products, particularly chocolate, and has been often investigated.

It was earlier concluded that more than 50% of the total glycerides of cocoa butter consists of one oleopalmitostearin, generally as a result of examination of hydrogenated fractions of cocoa butter. Amberger and Bauch [1] (also see Lewkowitsch [2]) found 57% of an oleopalmitostearin in cocoa butter. Amberger and Bauch [1] isolated a hydrogenated fraction of m. p. 63·5° and Lewkowitsch [2] concluded that this indicated the presence of 1-palmitodistearin (m. p. 65°) derived from 2-stearo-oleopalmitin or 2-oleopalmitostearin in the original cocoa butter. Hilditch and Stainsby's analysis [3] indicated oleopalmitostearin 52%, oleodistearin 19%, dioleostearin 12%, palmito-oleopalmitins 9%, oleodipalmitins 6%, and palmitostearins 2%. A more recent analysis by Meara [4] showed oleopalmitostearin 57%, oleodistearin 22%, oleodipalmitin 4%, dioleopalmitin 7%, dioleostearin 6%, triolein 1%, and saturated glycerides 3%. Both Hilditch and Stainsby [3] and also Meara [4] isolated, from hydrogenated fractions, material of m. p. 66—67° which they considered to consist largely of 2-palmitodistearin (m. p. 68°) derived from 2-palmito-oleostearin in the cocoa butter.

Lard is more variable in composition than cocoa butter. However, its glycerides are characterised by considerable contents of an oleopalmitostearin and dioleopalmitin. Thus a typical lard [5] contained oleopalmitostearin 34%, dioleopalmitin 40%, dioleostearin 5%, oleodipalmitin 9%, triolein 3%, and saturated glycerides 9%. Meara,[6] on the basis of the m. p., and Quimby, Wille, and Lutton [7] on the basis of *X*-ray data and cooling curves of hydrogenated lard fractions, were of the opinion that lard consists mainly of 2-palmitoglycerides.

In the present work the structure of the major glyceride of cocoa butter, and the major glyceride present in the disaturated glycerides of lard, have been re-investigated by cooling curves and *X*-ray and infrared analysis.

Experimental

The sample of cocoa butter used (I val., 36·0; sap. val., 194·4; free fatty acid, 0·7% as oleic) had the following component acids: saturated 62·4; oleic 33·2; linoleic 4·4. A second sample of cocoa butter (I val., 36·4; sap. val., 192·9; free fatty acid, 0·1% as oleic), used in several of the experiments, gave very similar results and led to the same conclusions.

Oleodisaturated Glycerides of Cocoa Butter.—A concentrate of the disaturated glycerides was

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[1] Amberger and Bauch, *Z. Untersuch. Nahr. Genuz.*, 1924, **48**, 371.
[2] Lewkowitsch, *J. Soc. Chem. Ind.*, 1933, **52**, 236T.
[3] Hilditch and Stainsby, *J. Soc. Chem. Ind.*, 1936, **35**, 95T.
[4] Meara, *J.*, 1949, 2154.
[5] Hilditch and Pedelty, *Biochem. J.*, 1940, **34**, 971.
[6] Meara, *J.*, 1945, 23.
[7] Quimby, Wille, and Lutton, *J. Amer. Oil Chemists' Soc.*, 1953, 186.

prepared by crystallisation from acetone. Cocoa butter (150 g.) was crystallised from acetone (10 ml./g.) at 0°; the solid fraction was recrystallised similarly; the two liquid fractions (I val., 50·1) comprised 26·9% of the original cocoa butter. The solid fraction from the second crystallisation was then recrystallised from acetone (10 ml./g.) at 15°, to yield fractions comprising 7·7% and 65·4% of the cocoa butter, and having I val. 28·3 and 31·5 respectively. The latter fraction contained a high proportion of the disaturated glycerides of the cocoa butter.

Oleodisaturated Glycerides of Lard.—A concentrate of the disaturated glycerides of lard (I val., 67·0; free fatty acid, 0·2% as oleic) was made by crystallising 895 g. from acetone (10 ml./g.) at 0°. The solid fraction (239 g.) was recrystallised from acetone (6 ml./g.) at 22·5° and the resulting solid (77 g.; I val., 24·5) was again recrystallised from acetone (6 ml./g.) at 23°. The last two liquid fractions (180 g.) were recrystallised from acetone (5 ml./g.) at 0°, to yield a fraction of I val. 35·0 (19% of the original lard), containing a high concentration of disaturated glycerides.

Preparation of 2-Oleodistearin, 2-Oleodipalmitin, 2-Oleopalmitostearin, 2-Palmito-oleostearin, and 2-Stearo-oleopalmitin.—For the synthesis of these glycerides, highly purified samples (see Table; theor. or recorded values in parentheses) of oleic, palmitic, and stearic acids were prepared by conventional means.

	Setting pt.	Acid value	Sap. value	I value	Linoleic [9] acid (%)
Palmitic ...	62·25° (62·6) [8]	218·3 (219·2)	218·4 (219·2)	0·1 (0·0)	—
Stearic	69·25 (69·4) [8]	197·8 (197·5)	197·3 (197·5)	0·0 (0·0)	—
Oleic	—	—	—	89·6 (89·9)	0·5 (0·0)

The palmitoyl and stearoyl chlorides required were prepared by treating the acids with thionyl chloride. They were rapidly distilled under a high vacuum, from a pre-heated bath. The distilled products contained *ca.* 1% of free fatty acid, as determined by Bauer's anilide method.[10] Oleoyl chloride, prepared [11] by treating oleic acid with thionyl chloride in benzene, was distilled as above and contained 1—2% of free acid.

(a) *2-Oleodistearin and 2-oleodipalmitin.* 1-Monostearin and 1-monopalmitin, prepared by Baer and Fischer's method,[12] contained 100% of monoglyceride as determined by the periodate method [13] and had m. p. 81·5° (cf. 81·5° [14]) and 77° (cf. 77° [14]), respectively.

1 : 3-Distearin and 1 : 3-dipalmitin were prepared by a similar method to that of Malkin.[15] The monoglycerides were treated with an equimolar amount of the acid chloride in pyridine–chloroform at 10—15° for 2 hr. The diglycerides were crystallised successively from alcohol and hexane at room temperature, to constant m. p. and hydroxyl value [1 : 3-distearin, m. p. 80·0° (cf. ref. 16), OH val. 89, I val. 0; 1 : 3-dipalmitin, m. p. 74·0° (cf. ref. 16), OH val. 96, I val. 0·0].

The diglycerides were refluxed with an excess of oleoyl chloride in pyridine–chloroform,[16] passed in light petroleum (b. p. 40—60°) (4 ml./g.) through alumina (twice wt. of glyceride; activated at 800°), eluted with light petroleum, and crystallised from acetone (15 ml./g.) at 0°. This removed traces of diglyceride and avoided the necessity for repeated crystallisation. 2-Oleodistearin and 2-oleodipalmitin had respectively m. p. 42·5°, 36·5° (cf. ref. 16), I val. 28·5, 30·2, and sap. val. 189·8, 202·8.

(b) *2-Oleopalmitostearin, 2-palmito-oleostearin, and 2-stearo-oleopalmitin.* 1-Palmito-3-stearin was prepared from 1-monopalmitin and stearoyl chloride as described above. 1-Oleo-3-stearin and 1-oleo-3-palmitin were prepared from 1-monostearin and 1-monopalmitin respectively by 1 mol. of oleoyl chloride in the same manner, except that the reaction temperature was allowed to rise to 30° (no appreciable formation of triglyceride); the products, crystallised at 0° from alcohol and then from hexane, had the properties tabulated (cf. refs. 17 and 18).

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[8] Francis and Piper, *J.*, 1939, 577.
[9] Hilditch, Patel, and Riley, *Analyst*, 1951, **76**, 81.
[10] Bauer, *Oil and Soap*, 1946, **23**, 1.
[11] Human and Mills, *Nature*, 1946, **158**, 877.
[12] Baer and Fischer, *J. Amer. Chem. Soc.*, 1945, **67**, 2031.
[13] Pohle, Mehlenbacher, and Cook, *Oil and Soap*, 1945, **22**, 115.
[14] Malkin and Shurbagy, *J.*, 1936, 1628.
[15] Malkin, *J.*, 1937, 1412.
[16] Craig, Lundberg, and Geddes, *J. Amer. Oil Chemists' Soc.*, 1952, **29**, 169.
[17] Verkade and van der Lee, *Rec. Trav. chim.*, 1936, **55**, 267.
[18] Daubert and Longenecker, *J. Amer. Chem. Soc.*, 1944, **66**, 53.

	M. p.	OH val.	I val.
1-Palmito-3-stearin	71·5°	93·5	0·0
1-Oleo-3-stearin	49	90	40·6
1-Oleo-3-palmitin	44	96	42·4

They were converted into triglycerides by treatment with the requisite acid chloride.[16] The products were purified as before by alumina and crystallisation from acetone, their properties being (cf. ref. 19):

	M. p.	I val.	Sap. val.
2-Oleopalmitostearin	37·5—38°	29·2	194·7
2-Palmito-oleostearin	40·5—41	29·4	195·1
2-Stearo-oleopalmitin	41—41·5	29·3	195·3

X-Ray diffraction data on the triglycerides are also tabulated. The spacings were in close agreement with the data recorded by Lutton.[20]

Synthetic Mixtures.—Synthetic mixtures (see Table) were made, of pure oleodisaturated glycerides, having compositions resembling approximately those of cocoa butter and of its constituent disaturated glycerides, based on Meara's [4] analysis of cocoa butter.

Mixture:	A	B	C	D	E
2-Oleopalmitostearin	55	—	—	69	—
2-Oleodistearin	21	21	21	26	26
2-Oleodipalmitin	4	4	4	5	5
2-Palmito-oleostearin	—	55	—	—	69
2-Stearo-oleopalmitin	—	—	55	—	—
Fr. of I val. 50·1 from cocoa butter	20	20	20	—	—

Cooling Curves.—The fat (15 g.), melted at 100°, was weighed into a $4\frac{1}{2} \times 1''$ test-tube and allowed to cooled to 41°; the tube was fixed in an air-bath maintained at 19°; the fat was then stirred by a gentle stroke of a ring stirrer every 15 sec., readings of the temperature being taken every minute. Stirring was continued until the sample was too stiff, generally before the maximum temperature (after supercooling) was reached.

X-*Ray Diffraction Spectra.*—A Metro-Vick *X*-ray unit with Cu-$K\alpha$ radiation was used, the sample-to-film distance being 12·5 cm. Only the more stable forms of the pure glycerides and fats were examined. Samples were normally prepared by slow crystallisation from a solvent, but it was more convenient in certain cases to use samples which had been stored in the solid state for a considerable time.

X-*Ray diffraction data.*

		Mixture									
Cocoa butter	Fract.[1]	A	B	C	D	E	POS[a] (β-3)	OPS[b] Sub. β'3	OPS[b] β'3	OSP[c] β'-2	Lard fract.[2]
					Short spacings (Å)						
5·45 s	5·36 m	5·41 s	—	5·30 mw	5·39 m	5·25 w	5·40 m	—	—	5·45 w	5·36 vw
5·20 w	5·11 w	5·11 m	—	5·08 vw	5·11 m	—	5·11 w	5·08 w	—	5·10 w	4·99 w
4·68 } vs	4·59 vs	4·63 } vs	4·70 s	4·85 vw	4·65 } vs	4·70 s	4·57 s	4·73 s	4·65 m	4·43 ms }	4·66 ms
4·54 }	—	4·53 }	4·57 s	4·60 s	4·52 }	4·47 } m	4·38 m	4·47 s	4·52 w	4·32 ms }	
4·28 w	4·27 w	4·24 vw	4·42 s	4·40 s	4·35 vw	4·32 }	4·22 m	4·27 } vs	4·41 w	4·15 ms }	4·13 vs
—	—	4·08 w	—	4·22 s	4·23 w	—	—	}	4·17 vs	4·03 ms }	
4·02 s	3·96 mw	3·97 s	4·20 s	3·92 s	3·98 m	4·15 } vs	4·02 ms	4·08 }	4·05 m	3·84 s }	
3·90 ms	3·85 mw	3·85 m	4·07 s	3·87 s	3·85 mw	4·05 }	3·89 w	3·84 s	4·00 m	3·67 w	—
3·79 ms	3·74 m	3·74 m	4·01 m	3·75 ms	3·72 mw	3·88 ms	3·84 w	3·74 s	3·80 ms	—	3·80 ms
3·68 s	3·66 m	3·65 s	3·88 w	3·60 w	3·65 s	3·77 ms	3·67 m	—	3·69 ms	—	3·72 ms
			3·80 s								
			3·70 s								
					Long spacings (Å)						
64·5	64·5	64·1	68·9	43·7	64·1	68·9	64·1	69·4	66·9	43·7	69·4

[1] Cocoa butter fraction of I value 31·5. [2] Lard fraction of I val. 35·7.
[a] 2-Oleopalmitostearin. [b] 2-Palmito-oleostearin. [c] 2-Stearo-oleopalmitin.

Infrared Spectra.—A Grubb-Parsons S.3 infrared spectrometer was used with a rock-salt prism, for the range 1800—650 cm.$^{-1}$, with capillary thicknesses of sample between rock-salt

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[19] Verkade, *Rec. Trav. chim.*, 1943, **62**, 393.
[20] Lutton, *J. Amer. Chem. Soc.*, 1951, **73**, 5595.

flats. The sample could be prepared as for the *X*-ray diffraction measurements, but for investigating polymorphic changes the rock-salt flats were enclosed in a cell the temperature of which could be varied.

RESULTS AND DISCUSSION

Cocoa butter, the crystallised cocoa butter fraction, and the crystallised lard fraction have been examined. Comparisons were made with pure samples of the three isomeric oleopalmitostearins and with synthetic mixtures of pure glycerides mixed in approximately the same proportions in which they occur in cocoa butter.

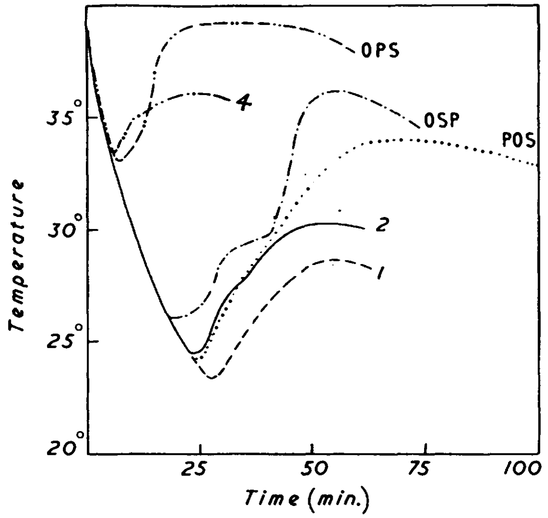


FIG. 1. *Cooling curves of pure glycerides, cocoa butter, and lard fraction.*

1, *Cocoa butter.*
2, *Cocoa butter fraction.*
3, *Synthetic mixture* A.
4, *Lard fraction.*
POS, 2-*oleopalmitostearin.*
OSP, 2-*stearo-oleopalmitin.*
OPS, 2-*palmito-oleostearin.*

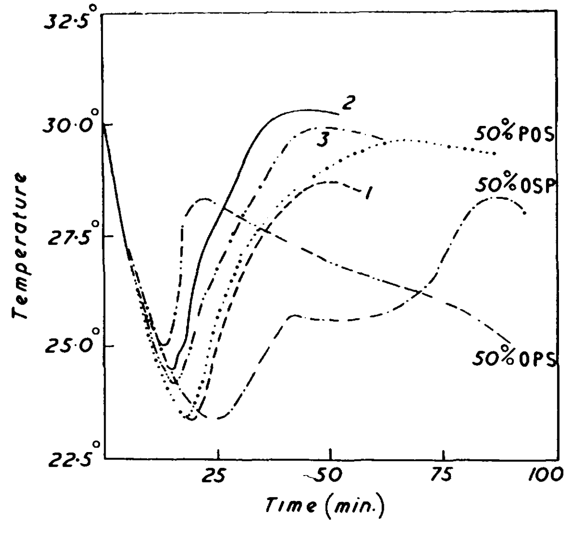


FIG. 2. *Cooling curves of mixtures of pure glycerides with cocoa butter.*

For key, see Fig. 1.

Cooling Curves.—The cooling curve of a fat or fat fraction is frequently of assistance in determining the nature of the glycerides present (cf. Quimby *et al.*[7]). The curves for cocoa butter and the cocoa butter fraction of I value 31·5 have been compared with those for the three oleopalmitostearins (Fig. 1) and 1 : 1 mixtures of each of these glycerides with cocoa butter (Fig. 2).

The curve for 2-oleopalmitostearin closely resembles that for cocoa butter and the cocoa butter fraction, whereas the curves for 2-stearo-oleopalmitin and particularly for

FIG. 3.

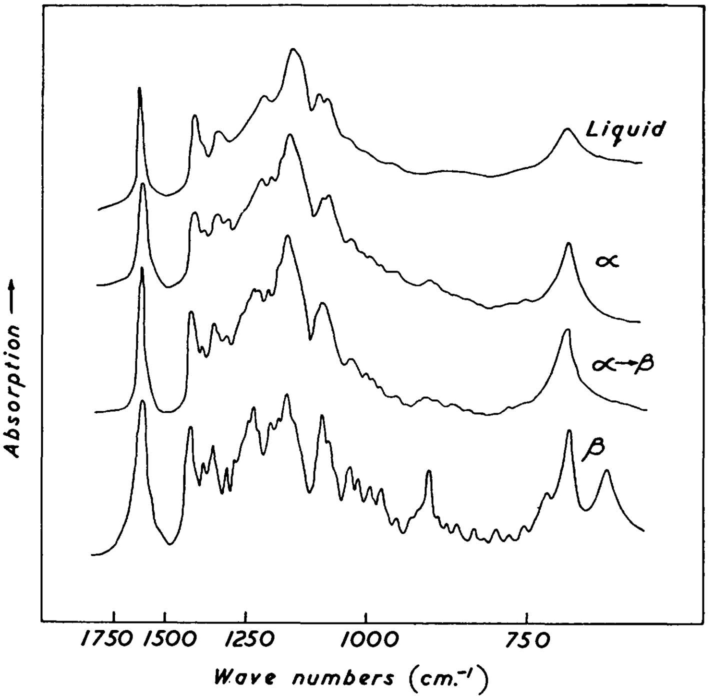
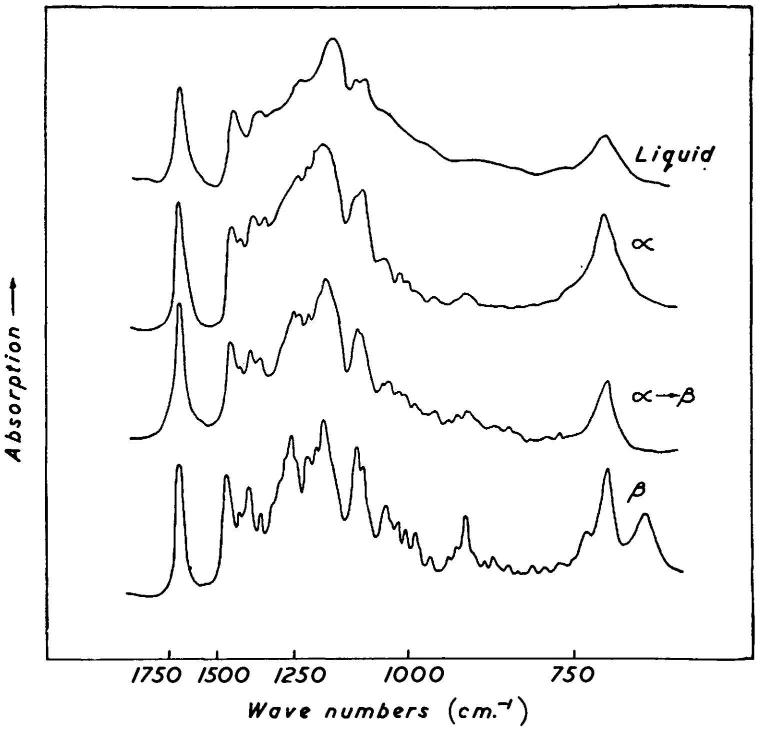


FIG. 4.



FIGS. 3 & 4. *Infrared spectra of polymorphic forms of (Fig. 3) cocoa butter and (Fig. 4) mixture* A.

FIG. 5.

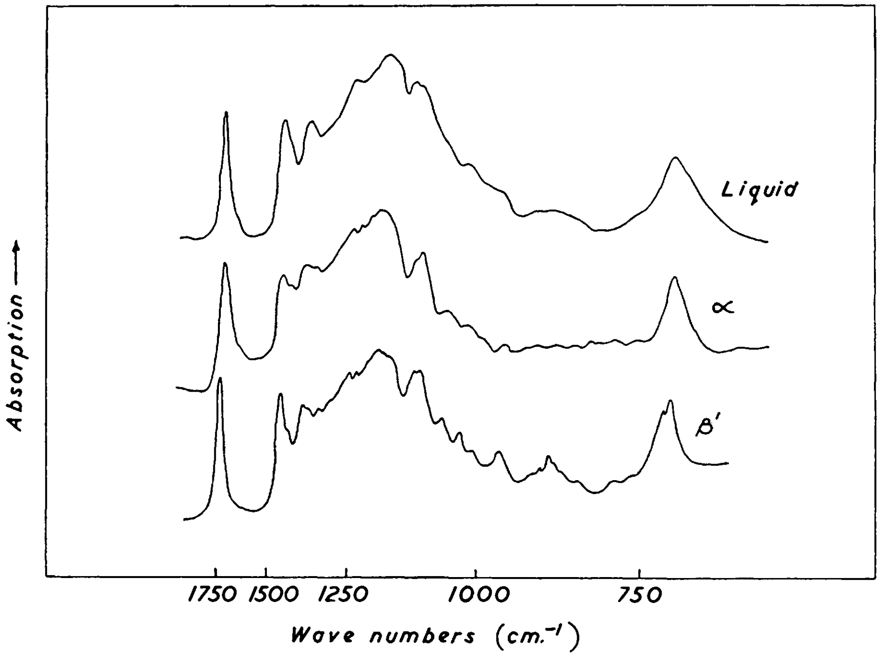
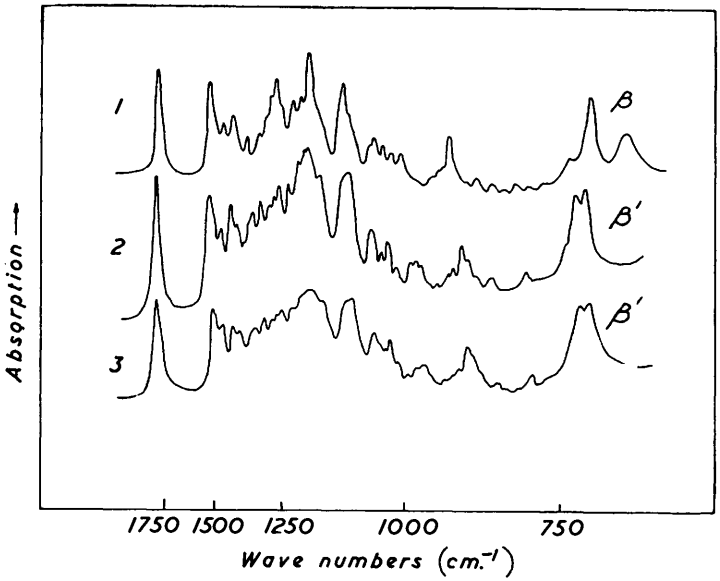


FIG. 6.



FIGS. 5 & 6. *Infrared spectra of* (*Fig.* 5) *polymorphic forms of mixture* B, *and* (*Fig.* 6) (1) 2-*oleopalmitostearin,* (2) 2-*palmito-oleostearin, and* (3) *a lard fraction.*

2-palmito-oleostearin are characteristically different. This is a strong indication of the presence, in cocoa butter, of the 2-oleo-isomer; it is confirmed by the cooling curves of 1 : 1 mixtures of the three glycerides with cocoa butter (Fig. 2). The inflexion in the curve of 2-stearo-oleopalmitin is magnified when this glyceride is mixed with cocoa butter; and the mixture containing 2-palmito-oleostearin has a smaller rise of temperature after supercooling than has cocoa butter. In the latter case, although the curve superficially resembles that for cocoa butter, this mixture is characteristically different from cocoa butter and the other 1 : 1 mixtures, in remaining semiliquid and stirrable for a considerable time (57 min.) after the maximum temperature has been reached. The curve for 2-oleopalmitostearin, however, is similar to that of cocoa butter and the cocoa butter fraction, again indicating the presence of the 2-oleo-isomer. In order to confirm that the remaining glycerides of cocoa butter do not alter the character of the cooling curve of the 2-oleo-compound, the cooling curve for synthetic mixture A (containing the liquid glycerides o cocoa butter) was determined (Fig. 2) : it is also similar to that for cocoa butter.

The cooling curve of the lard fraction of I value 35·0, which is known to contain a preponderance of one of the isomers of oleopalmitostearin, is unlike that for cocoa butter and is very similar to the curve for 2-palmito-oleostearin.

X-Ray Investigation.—Cocoa butter is known to exhibit polymorphism. An *X*-ray powder photograph of cocoa butter in its most stable form gave a pattern analogous to that obtained with pure 2-oleotriglycerides in their most stable form (designated β-3 for the pure glycerides investigated by Lutton [20]). The pure 1-oleotriglycerides have a different *X*-ray pattern and stable form (designated β′-3 by Lutton). This evidence alone suggests that the major glyceride in cocoa butter is the 2-oleo-isomer.

The *X*-ray patterns (see Table) of cocoa butter, the cocoa butter fraction, and of the synthetic mixtures containing the 2-oleo-isomer, are very similar to each other and also resemble that of the pure 2-oleo-isomer. There is little or no resemblance to the patterns of the 2-stearo- or 2-palmito-glycerides, or of synthetic mixtures containing these glycerides.

Infrared Spectroscopic Investigation.—Chapman [21] showed that the polymorphic forms of glycerides and natural fats can be characterised by their spectra, and that, as well as being indicative of a particular polymorphic form, the spectra can also provide information on the chain length and the molecular groupings present. The infrared spectra of some of our materials, in various polymorphic forms, are shown in Figs. 3—5. The spectrum of the most stable polymorphic form (a β′-form) of mixture B differs from the spectra of cocoa butter. The spectra of mixture C (containing the 2-stearo-isomer as the predominant glyceride) are not shown but were also dissimilar from those of cocoa butter. The spectra of mixtures D and E (containing no liquid glycerides) were similar to those of mixtures A and B respectively.

It is of interest that the spectrum of cocoa butter in its most stable crystalline form is very similar to that of the pure 2-oleo-isomer in its β-3 form. (The spectra of the pure glycerides will be described elsewhere.)

The infrared spectrum of the stable polymorphic form of a lard fraction is shown in Fig. 6 : in contrast with that of cocoa butter, it is very similar to that of the 2-palmito-isomer in its stable crystalline form, consistently with the conclusions of Meara [6] and of Quimby *et al.*[7] that the oleodisaturated glycerides of lard consist mainly of this glyceride.

The results from the three methods, *viz.*, cooling curves, *X*-rays, and infrared spectroscopy, show that the main glyceride present in cocoa butter is 2-oleopalmitostearin, and not 2-palmito-oleostearin as previously suggested. In fact 2-palmito-oleostearin has been shown to be the main disaturated glyceride present in lard.

This work has shown the desirability of using several independent analytical methods, in order to arrive at a definite conclusion regarding glyceride configuration. The difficulties of structural analysis of glycerides, as pointed out by Hilditch,[22] mainly occur in the

[21] Chapman, *J.*, 1956, **55**; VIth Internat. Spectroscopic Colloquium, Amsterdam, 1956.

[22] Hilditch, "The Constitution of the Natural Fats," Chapman and Hall, London, 1956.

isolation of pure glycerides from natural fats. However, much information may be gained by application of the techniques, described in the present paper, to concentrates of glycerides and of their hydrogenated products, obtained by low-temperature crystallisation or by other means. By these methods it should also be possible to determine the structures of many other natural fats, as of the minor constituent glycerides of cocoa butter and of the diunsaturated glycerides of lard.

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