330. *The Interconversion of* cis- *and* trans-*Modifications of Monoethylenic Higher Fatty Acids by Nitrogen Trioxide (the "Elaidin" Reaction).*

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IN the investigation of mixed glycerides present in natural fats, quantitative methods are at present only available for the determination of those which contain three saturated acyl radicals, and attention is therefore being directed in these laboratories to procedures which might lead to the estimation of other types of triglycerides, especially those containing only unsaturated acyl radicals. We have examined, in this connexion, the conversion of triolein into trielaidin in presence of oxides of nitrogen, an action which was apparently first observed by Poutet in 1819 (Boudet, *Annalen*, 1832, **4**, 1; Varrentrapp, *ibid.*, 1840, **35**, 196) and was formerly much used as a qualitative test for "non-drying" (*e.g.*, olive or almond) oils (Archbutt, *J. Soc. Chem. Ind.*, 1886, **5**, 303). In spite of its antiquity, and although Farnsteiner (*Z. Unters. Nahr. Genussm.*, 1899, **2**, 1) attempted to employ it in the determination of oleic acid in the mixed acids of butter and other fats, yet little information of a quantitative nature has been given with reference to the equilibrium established between *cis*- and *trans*-forms in the "elaidin" reaction, except that Jegorow (*J. Russ. Phys. Chem. Soc.*, 1903, **35**, 973; *J. pr. Chem.*, 1912, **86**, 539) observed that the transformation was effected by relatively small proportions of the reagent and that the use of larger proportions enabled him to isolate addition products of the composition $C_{18}H_{34}O_2(NO_2)(NO)$ and $C_{18}H_{34}O_2(NO_2)(OH)$.

It was thus necessary to study, from a quantitative point of view, the interconversion of the fatty acids before proceeding to the more complex case of triolein, and the present paper is chiefly concerned with the isomerisation of ordinary oleic ($\Delta^{9:10}$-octadecenoic),* petroselinic ($\Delta^{6:7}$-octadecenoic), and erucic ($\Delta^{13:14}$-docosenoic) acids

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* In order to correlate the higher fatty acids with the corresponding alcohols, the carboxylic carbon atom is numbered 1 in defining the position of the ethenoid bond, as in the Geneva notation.

and their respective *trans*-isomerides; the isomerisation of methyl oleate and of triolein was subsequently compared with that of oleic acid.

Isomerisation of Oleic Acid by Oxides of Nitrogen.—The action of oxides of nitrogen, prepared by different methods, on oleic acid was first studied. The products were separated by means of the difference in solubility of lead oleate and elaidate (cf. p. 2320), and the amount of octadecenoic acid in each part was estimated by determination of the iodine absorption (Wijs). In all cases the acids from the soluble lead salts, and (in much less degree) those from the insoluble lead salts, possessed lower iodine values than that of pure oleic or elaidic acid; qualitative tests showed the presence of combined nitrogen in both fractions of acids (although not in marked quantity in the elaidic fractions). It was evident that the final product of the action consisted of both *cis*- and *trans*-forms of the octadecenoic acid accompanied by varying proportions of addition products of oxides of nitrogen with the ethenoid acids. The experiments were therefore carried out with the aim of reaching complete equilibrium, and the results were compared on the basis of the amount of elaidic acid in the product calculated as a percentage on the oleic acid originally employed; the difference between the latter and the sum of the elaidic and oleic acids recovered (as indicated by iodine value) is a measure of the amount of addition products present in the equilibrium mixtures.

Poutet's reagent (mercury dissolved in nitric acid, *d* 1·42), as recommended by Archbutt (*loc. cit.*), was usually found to give high yields (65—67%) of elaidic acid with lower proportions of addition products than the use of gaseous oxides of nitrogen, except when the latter were prepared from arsenious oxide and nitric acid; but the nitrous fumes obtained by the action of sulphuric acid upon sodium nitrite generally effected almost as large a conversion into elaidic acid (56—60%), although in this case the proportions of permanent addition compounds were notably larger. The oxides of nitrogen evolved in the action of nitric acid (*d* 1·42) upon copper, however, gave a yield of only 25% of elaidic acid, with about 67% of the original oleic acid in the form of addition products (as measured by non-reactivity with the Wijs reagent).

The results, as a whole, led to the conclusion that, under the most favourable conditions, oleic acid was converted at equilibrium into about 66% of its weight of elaidic acid. The conditions favouring this result include the use of minimal amounts of oxides of nitrogen approximating in composition to N_2O_3 at 10—20°; a higher temperature, or excess of the reagent, leads to the presence of greater amounts of addition compounds in the final product.

Isomerisation of Oleic Acid by Sulphur.—Rankow (*Ber.*, 1929, **62**, 2712) has recently reported the partial transformation of oleic into elaidic acid at about 200° in presence of small amounts of sulphur, a method somewhat similar to that of M. C. and A. Saytzew (*J. pr. Chem.*, 1894, **50**, 73). We have analysed products obtained according to Rankow's method by the same procedure as in the above instances and find that the conversion into elaidic acid is of the order of 56—60%, with only 2—3% of addition products (indicated by iodine value determinations); combined sulphur was present, as stated by Rankow.

It appeared, especially as it was desired to apply the process to glycerides as well as to the free acids, that the use of oxides of nitrogen in one of the forms leading to a minimum of addition products was to be preferred, and, in the remaining experiments, the reagent employed was either Poutet's solution or the gases produced from sulphuric acid and sodium nitrite.

It seemed desirable, whilst engaged in a quantitative study of the "elaidin reaction," to investigate the reverse isomerisation of elaidic acid and also corresponding isomeric changes of some other monoethylenic acids of high molecular weight.

Isomerisation of Elaidic Acid.—The mercury–nitric acid reagent led to the production of a mixture containing elaidic acid in amount equal to about 68% of that submitted to the process, whilst nitrous fumes (*a*) from arsenious oxide and nitric acid, and (*b*) from sodium nitrite and sulphuric acid led respectively to the recovery of 66% and 60% of the elaidic acid employed in the isomerisation. As with oleic acid, the proportions of recovered acids which did not react with the Wijs reagent (*i.e.*, addition products) were greatest with oxides of nitrogen generated from sodium nitrite and least in the case of the Poutet reagent.

It will be seen from the experimental tables that the yields of elaidic acid, starting from either oleic or elaidic acid, are closely similar when the same method of preparation of oxides of nitrogen is employed, and we regard this as formal proof (hitherto, we believe, lacking) that the "elaidin reaction" is a reversible change, the same equilibrium being reached irrespective of whether the *cis-* or the *trans-*acid is the starting point.

Isomerisation of Petroselinic (cis-$\Delta^{6:7}$-*Octadecenoic*) *and of Erucic* (cis-$\Delta^{13:14}$-*Docosenoic*) *Acids.*—Each of these acids, when submitted to the Poutet reagent, gave yields of about 60% of the corresponding *trans-*acids. The proportion of the latter at equilibrium was thus in each case somewhat less than in the elaidinisation of oleic acid by the same reagent, and at the same time the presence of addition products was somewhat more noticeable. There appears to be some relationship between the m. p. of the original acid and the proportion of unresolved addition products in the equilibrium mixture, judging from the respective figures for oleic, petroselinic, erucic, and elaidic acids with the Poutet reagent. These varying proportions of addition products render it difficult to attempt to draw conclusions as to any relations which may exist between the composition of the *cis-trans* mixture at equilibrium and the constitution of the three monoethylenic acids examined.

Isomerisation of Methyl Oleate.—Before proceeding to study triolein, it was felt desirable to compare the equilibrium attained in the case of a simple ester with that for oleic acid. It was found that the Poutet reagent converted methyl oleate to the extent of 66% into methyl elaidate with formation of addition products in much the same quantity as in the case of oleic acid, and therefore it was inferred that the process operated in quantitatively the same manner with an ester as with the free acid.

Isomerisation of Triolein.—The quantity of trielaidin isolated in three experiments in which triolein was treated with the mercury–nitric acid reagent was 26—31% of the triolein taken. At the same time, the mixed fatty acids from the isomerised glyceride were found to contain 61—66% of elaidic acid, so that at equilibrium the same total ratio of elaidic to oleic acid obtained as in the isomerisation of oleic acid itself or of methyl oleate. As would be expected, therefore, the number of triolein molecules completely converted into trielaidin at equilibrium is only about 30% of the whole, the remainder consisting of mixed oleo-elaidins and (possibly) unaltered triolein. If we take the proportions, in round numbers, of trielaidin and total elaidic derivatives as respectively 30% and 65%, it follows that the remaining 70% of mixed oleo-elaidins may contain (i) about 35% each of mono-oleo-dielaidin and dioleo-monoelaidin, (ii) about 52·5% of mono-oleo-dielaidin and about 17·5% of unaltered triolein, or (iii) all three glycerides in amounts falling within these limits.

The probable composition of a mixture of triglycerides produced from triolein by conversion of 65% of the oleic into elaidic radicals, deduced by algebraical reasoning, would be 30% trielaidin, 44% dielaido-mono-oleins, 22% monoelaido-dioleins, and 4% triolein. Since the observed proportion of trielaidin is not far removed from that estimated on this basis, it is quite likely that the remaining components are also present in similar quantities to those suggested by arithmetical considerations.

For determination of triolein in natural fats, however, the " elaidin reaction " is shown by these experiments to be of doubtful utility. If triolein alone had to be considered, it is probable that the procedure would be of at least semi-quantitative value, in spite of the production of so small a proportion of trielaidin as 30% of the triolein present. Unfortunately, when triolein is a component of fats, it is practically always accompanied by considerable quantities of mixed saturated-unsaturated glycerides such as palmito-oleins; and the concurrent partial transformation of the latter into corresponding palmitoelaidins (which would separate with trielaidin) would appear to render the determination of trielaidin itself very difficult in the majority of cases.

Although the present investigation has thus indicated that the "elaidin reaction" is not likely to be of great service as a means towards the estimation of triolein, the following conclusions have been reached:

1. The process is an equilibrium action and the composition of the final product is the same whether the *cis*- or the *trans*-ethenoid acid is isomerised. The *trans*-acid is formed to the extent of about 66% (oleic, elaidic) or somewhat less (petroselinic, erucic) of the acid isomerised; the yield varies somewhat according to the method of preparation of the oxides of nitrogen, and also, probably, with acids of different constitution.

2. Definite amounts of addition products accompany the mixture of *cis*- and *trans*-acids at equilibrium, and it is to be inferred that the isomeric change is effected by addition of nitro- and nitroso-radicals at the ethenoid bonds, followed by reversion of the addition compound into nitrogen trioxide and the equilibrium mixture of *cis*- and *trans*-compounds.

3. The action proceeds to quantitatively the same extent as in the free acid in the methyl or glyceryl esters of oleic acid; but, owing to the presence of three oleic groups in triolein, only about 30% of the latter is completely transformed into trielaidin.

4. The present investigation has indicated the possibility that semi-quantitative application of the "elaidin reaction" may prove useful in the following directions: (i) the approximate estimation of oleic acid in mixtures of oleic with linoleic and other unsaturated acids present in certain classes of natural fats, especially in the "drying" oils; (ii) the isolation, by crystallisation from acetone (see p. 2323), of mixtures of trielaidin and palmitoelaidins from different natural fats in characteristic proportions and with characteristic equivalents and iodine values, thus giving a certain quantitative significance to what was formerly only a qualitative test.

These extensions of the procedure will be described in detail in due course.

EXPERIMENTAL.

The oleic acid was prepared from the mixed acids of olive oil: most of the satd. acids present in the latter were first removed in the form of Pb salts insol. in EtOH, and the unsatd. acids were then converted into Me esters and fractionally distilled, the earlier portions of the distillate being rejected and only those free from methyl palmitate being reserved. The latter were reconverted into acids, the Li salts of which were crystallised several times from 80% EtOH; the oleic acid obtained from the purified Li salts (I.V. 90·4) was almost pure and did not contain more than 0·5% of palmitic acid.

Petroselinic acid was obtained from the distilled fractions of its Me ester which were isolated in former work on various seed-fats of the *Umbelliferæ* (Christian and Hilditch, *Biochem. J.*, 1929, **23**, 327), and was recrystallised from EtOH several times before use (m. p. 30°, I.V. 89·0).

Erucic acid was prepared by fractional distillation of the Me esters of the unsatd. acids of rape oil, followed by crystn. of the resulting acid from EtOH (m. p. 33·4°, I.V. 74·7).

Analytical Estimation of the trans-*Ethenoid Acids.*—(i) *Elaidic in presence of oleic acid.* In this case advantage can be taken of the difference in solubility in EtOH, under specified conditions, of the Pb salts: we employed the procedure of Cocks, Christian, and Harding (*Analyst*, 1931, **56**, 368), who wash the Pb salts deposited from 93% (wt.) EtOH with light petroleum (b. p. 40—60°) to remove any retained Pb oleate, and subsequently again crystallise from EtOH the salts removed by light petroleum. The fatty acids from the Pb salts separated in the original and the subsidiary alcoholic crystns. are weighed and their I.V. determined. This method was tested on mixtures of oleic and elaidic acids in known proportions, with the following results:

Elaidic acid in mixture, %	100	79·8	61·4	40·9	33·2	17·2
,, ,, found, %	97·3	82·0	64·3	48·6	41·5	15·4

Thus, although the process is not reliable when elaidic acid forms the minor component of the mixture, it yields results within 2—3 units of the actual figures over a range which, fortunately, includes the composition of nearly all of the isomerised mixtures encountered during the present work.

(ii) trans-$\Delta^{6:7}$-*Octadecenoic in presence of petroselinic acid, and brassidic in presence of erucic acid.* Pb petroselinate and Pb erucate are both only sparingly sol. in EtOH, and therefore the estimation of the respective *trans*-acids could not in these cases be effected simply by the method described above. It was found, however, that a preliminary crystn. of the Pb salts of the acids after isomerisation was useful, because the Pb salts of unresolved addition compounds were always found to be relatively sol. in EtOH and were thus removable from the *trans*-acid Pb salts. The composition of the acids recovered from the insol. Pb salts (mixtures of the *trans*-acid with a certain amount of unchanged *cis*-acid) was approx. determined by recourse to the m. p. curves for mixtures of the *cis*- and *trans*-acids concerned. These were determined for each pair of acids with the results shown in the figure.

In all cases the wts. of acids recovered after Pb salt separation were converted, according to the observed I.V.'s, into the corresponding wts. of the unsatd. acid in question; the I.V.'s of the pure acids were: oleic 90·1, elaidic 88·8, petroselinic (*cis*- and *trans*-forms) 89·0, erucic and brassidic 74·7.

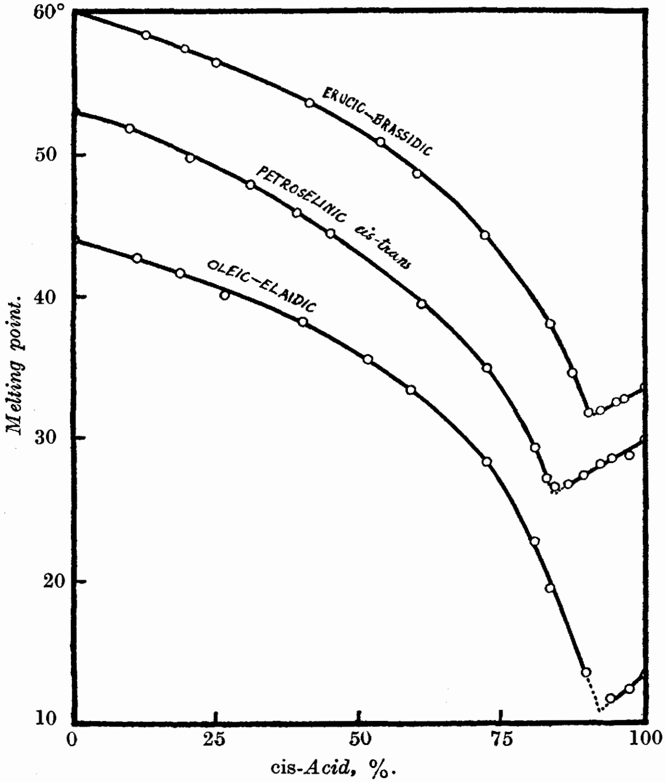
Quantitative Data obtained in the Isomerisation of the Four Acids.—A selection of the experimental results is given in Table I, in which the precise method of effecting the reaction is indicated as follows:

"*P.*" 1 Part of Poutet's Hg–HNO_3 reagent (prep. by dissolving 6 g. of Hg in 15·6 c.c. of HNO_3, *d* 1·42) was shaken with 12 parts of the fatty acid or ester in a stoppered vessel immersed in H_2O at room temp. for 1 hr. and left over-night.

" *S*." Oxides of N generated from $NaNO_2$ and H_2SO_4 were passed into a flask, containing a weighed quantity of oleic or other acid, which was well shaken for about 15 mins. and then stoppered and set aside over-night.

" *A*." As " *S*," but with oxides of N from As_2O_3 and HNO_3.

" *C*." As " *S*," but with oxides of N from Cu and HNO_3.



The results obtained on agitating oleic acid with 1% of S at 220° for 3 hrs. in presence of air, SO_2, or CO_2 are similarly shown in Table II. It was obs. that the I.V.'s of the products frequently exceeded that of oleic or elaidic acid, if the Wijs reagent was left in contact with the acids for more than 30 mins.; this abnormal behaviour probably indicates that S derivs. of the fatty acids may interact with the Wijs reagent, and consequently the derived values for elaidic and oleic acids may be somewhat too high. The low m. p.'s of the acids from the insol. Pb salts also suggest the presence of impurities.

Isomerisation of Methyl Oleate.—Methyl oleate (6·1 g.), after treatment with the Hg–HNO_3 reagent, was hydrolysed, and the mixed fatty acids were submitted to the Pb salt separation. The acids from the insol. salts (I.V. 86·9) amounted to 67·5% of the oleic acid present in the original ester, whilst the I.V.

TABLE I.

Isomerisation of monoethylenic higher fatty acids by oxides of nitrogen.

Acid employed.			Septn. of products by Pb salts.				Comp. of product, %.		
	g.	Method.	Acids from insol. salts. %.	M. p.	I.V.	"Liquid" acids. I.V.	*trans*-Acid.	*cis*-Acid.	Addition products.
Oleic	34·3	P	68·0	41·5°	85·8	82·7	66·7	29·4	3·9
,, ...	16·7	,,	72·6	42	79·2	45·0	65·8	13·4	20·8
,, ...	12·2	A	73·2	41	81·7	42·6	68·4	13·0	18·6
,, ...	21·8	S	61·9	42	84·9	51·1	60·0	20·0	20·0
,, ...	8·2*	,,	62·8	40·5	78·6	20·7	58·2	9·0	32·8
,, ...	9·8	C	29·8	40	73·2	10·4	24·6	8·1	67·3
Elaidic	3·5	P	73·2	41	83·8	44·3	68·7	13·3	18·0
,, ...	3·5	,,	70·8	43	85·5	53·1	68·6	16·6	14·8
,, ...	3·8	A	67·4	41	84·2	17·6	66·7	6·7	26·6
,, ...	3·5	S	59·4	42	85·6	15·1	60·4	6·8	32·8
Petroselinic ...	17·8	P	71·2†	51	83·5	51·2	61·2	19·6	19·2
,, ...	5·2	,,	66·7	51·2	88·0	44·3	58·9	21·8	19·3
Erucic	8·2	P	71·4	58·4	71·2	37·2	62·8	20·0	17·2
,, ...	5·2	,,	64·2	57·6	67·6	20·7	54·2	21·5	24·3

* This expt. was carried out at 0°, all the remainder at 18—20°.

† On recrystn. from EtOH, these acids yielded (i) 66·3%, m. p. 53°; (ii) 21·3%, m. p. 50·5—51·5°; and (iii) 12·4%, m. p. 30—32°.

TABLE II.

Isomerisation of oleic acid by 1% *sulphur at* 220°.

	Septn. of products by Pb salts.				Approx. comp. of products, %.		
	Acids from insol. salts.			"Liquid" acids.			
Atmosphere.	%.	M. p.	I.V.	I.V.	Elaidic acid.	Oleic acid.	Addition products (?).
Air	55·7	36°	86·2	87·7	54	43	3
,,	60·8	36	88·8	86·0	61	35	4
SO_2	56·5	31	86·6	89·7	56	43	2
CO_2	58·9	35	87·9	88·6	59	39	2

of the "liquid" acids was 75·1; this corresponds to the presence of 66·2% elaidic and 26·3% oleic acid, calc. on the oleic acid originally present, together with 7·5% of unresolved addition products.

Isomerisation of Triolein.—(i) *Preparation of triolein and trielaidin.* Glycerol was heated with excess of the fatty acid at 130° for 6 hrs. in vac.; a small quantity (0·4%) of naphthalene-β-sulphonic acid was added to the mixture to promote esterification. The neutral glycerides thus obtained were resubmitted to esterification under similar conditions with more of the fatty acid to ensure complete conversion of the product into triglycerides. The characteristics of the triolein and trielaidin obtained were as follows :

		Trielaidin,	
	Triolein, found.	found.	calc.
I.V.	85·2	85·2	86·2
Sap. equiv.	293·9	294·1	294·7
Acetyl val.	1·3	3·2	nil

The trielaidin melted at 42·8°, and separated from CMe_2O in wart-like clusters of crystals; the solubility in CMe_2O (100 c.c.) at 0° was 0·29 g., and at 20°, 0·98 g.

(ii) *Estimation of trielaidin in presence of triolein.* Practically complete separation of trielaidin from mixtures of triolein and trielaidin could be effected by crystallising the mixture of glycerides (5 g.) from CMe_2O (20 c.c.) at room temp. over-night, followed by cooling at 0° for 2 hrs.; the separated crystals were collected and washed four times with CMe_2O (5 c.c.). The following results were obtained :

Trielaidin present,	%	100·0	78·6	61·2	39·4	19·6
Trielaidin isolated	%	99·3	78·4	61·0	39·2	18·3
	m. p.	42·3°	42·1°	42·2°	42·0°	42·3°

(iii) *Action of Poutet's reagent on triolein.* The isomerisation was carried out as described for oleic acid, but the product was divided into two portions for examination :

(*a*) One portion (5 g.) was crystallised from CMe_2O under the above conditions; the separated crystals were weighed and their m. p. determined, whilst the material in the mother-liquor and washings was recovered and its I.V. estimated. The results are shown below :

Isolation of trielaidin from isomerised triolein.

Triolein used, g.	Products septd. from acetone.			Sol. glycerides.	Tri-elaidin,	Apparent addition products,
	%.	M. p.	I.V.	I.V.	%.	%.
18·0	32·6	40·5°	81·4	50·7	31·1	29·2
9·4	26·7	40·5	82·9	61·8	26·3	21·0
8·8	28·5	41·5	83·4	61·7	28·0	20·7

The m.p. of the cryst. product was in each case but little below that of trielaidin, and was raised somewhat on admixture with it. Dielaido-monooleins, therefore, do not separate in the solid state under the conditions employed; the slight depression in m. p. is amply accounted for by the presence of traces of addition products which are indicated by the correspondingly slightly lower I.V.'s (trielaidin, I.V., obs., 85·2).

(*b*) The remainder of the isomerised triolein was hydrolysed by alc. KOH, and the resulting mixture of acids was submitted to the Pb salt separation as described in previous cases : the total conversion of oleic into elaidic radicals in the mixed glycerides was thereby determined (see below).

Total conversion of oleic to elaidic radicals in isomerised triolein.

Triolein used, g.	Septn. of products by Pb salts. Acids from insol. salts.		"Liquid" acids.	Approx. comp. of products, %.		
	%.	I.V.	I.V.	Elaidic acid.	Oleic acid.	Addition products.
18·0	68·2	79·5	54·5	61·1	19·2	19·7
9·4	71·1	83·2	57·1	66·6	18·3	15·1
8·8	68·6	83·1	59·4	64·2	20·7	15·1

These analyses show that the same equilibrium is reached (between oleic and elaidic acid radicals as a whole) in triolein as in methyl oleate or oleic acid. They also indicate that, to some extent, unresolved addition products present in the isomerised products are reconverted into unsatd. acids by the action of hot alc. alkali.

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