686. *The Constitution of Vaccenic Acid.*

By S. S. GUPTA, T. P. HILDITCH, S. PAUL, and R. K. SHRIVASTAVA.

The traces of *trans*-octadecenoic acids present in the body and milk fats of oxen and sheep, and termed " vaccenic acid," contain both *trans*-octadec-10-enoic and *trans*-octadec-11-enoic acids.* Other fatty acids so far recorded in natural fats are invariably *cis*-isomers. The appearance of traces of *trans*-isomers in certain animal fats suggests that these may be the result of metabolic alteration of the natural (*cis*-)glycerides in the specific animals concerned, leading to both *cis*- and *trans*-forms of the artefacts in question.

VACCENIC ACID, the name assigned by Bertram (*Biochem. Z.*, 1928, **197**, 433) to an acid present in very small proportions (0·1 to at most 1%) in ox and sheep body fats and in butter-fat, was stated by him to be *trans*-octadec-11-enoic * acid. In recent years the acid has attracted some attention owing to reports (Boer and Jansen, *Arch. néerl. Physiol.*, 1941, **26**, 1; Boer, Jansen and Kentie, *J. Nutrition*, 1947, **33**, 339, 359) that it conferred growth-promoting properties on butter-fat; but these findings were not confirmed by other workers (Euler and Euler, *Z. Vitaminforsch.*, 1948, **1**, 474; Deuel and Greenberg, *J. Nutrition*, 1948, **35**, 301) or by later observations of Boer, Groot, and Jansen (*Voeding*, 1948, **9**, 60). In connection with these nutritional studies, Groot, Kentie, and Knol (*Rec. Trav. chim.*, 1947, **66**, 633) had recourse to the preparation of presumed *trans*-octadec-11-enoic (vaccenic) acid by the partial hydrogenation of the elæostearic glycerides in tung oil (as described earlier by Boeseken, Krimpen, and Blanken, *ibid.*, 1930, **49**, 247). Recent observations have created some doubt as to whether either Bertram's natural vaccenic acid or the hydrogenated product from elæostearic acid in fact possesses the constitution of an individual octadec-11-enoic acid.

Ahmad, Bumpus, and Strong (*J. Amer. Chem. Soc.*, 1948, **70**, 3391) synthesised *cis*-octadec-11-enoic acid (m. p. 10·5—12°) from 1-chloroheptadec-10-yne and obtained from it by isomerisation with selenium *trans*-octadec-11-enoic acid, m. p. 43—44° [the structure of the *trans*-acid was verified by Bumpus, Taylor, and Strong (*ibid.*, 1950, **72**, 2116) by its oxidation to 11 : 12-dihydroxystearic acid and scission of the latter by periodic acid]. Ahmad *et al.* found that the infra-red spectrum of a specimen of highly-purified natural vaccenic acid (m. p. 42·5°) prepared from beef tallow by Rao and Daubert (*ibid.*, 1948, **70**, 1102) was identical with that of the synthetic *trans*-octadec-11-enoic acid. On the other hand, Benedict and Daubert (*ibid.*, 1949, **71**, 4113) studied the *X*-ray diffraction spectra of elaidic (*trans*-octadec-9-enoic), the synthetic *trans*-octadec-11-enoic, and the specimen of natural vaccenic acid mentioned (m. p. 42·5°) : the long and side spacings of elaidic and the synthetic *trans*-octadec-11-enoic acids were identical, whereas those of the natural vaccenic acid were markedly different. Their conclusion that the dissimilarities in the *X*-ray spectra may possibly be due to differences in the structure of the acids is somewhat difficult to accept, for, since isomers with a *trans*-ethenoid bond in the 9 : 10- and 11 : 12-positions give identical spectra, it is not easy to understand why a *trans*-ethenoid bond in another position in the molecule should give rise to different spacings. It is perhaps more readily conceivable that a mixture of *trans*-acids with the double bond in different positions might lead to the observed differences in the *X*-ray diffraction spectrum.

We ourselves have, from time to time in the past 12—15 years, had occasion to examine natural vaccenic acid concentrates from various sources. We have verified most of Bertram's original observations, with one exception : we have consistently failed to identify *n*-heptanoic acid and nonanedicarboxylic acid, $[CH_2]_9(CO_2H)_2$, as the sole products of disruptive oxidation of vaccenic acid. We have, on the contrary, found evidence of the concurrent presence in these products of *n*-octanoic and sebacic acids (sometimes in relatively large proportions), thus indicating the presence of an octadec-10-enoic acid in the material submitted to oxidation. In view of the recent observations by the American workers (*loc. cit.*) on infra-red and *X*-ray diffraction spectra of vaccenic acid and related acids, we believe that a brief record of the chemical evidence which we have obtained over a period of some years in regard to the structure of vaccenic acid may be of interest.

Bertram (*loc. cit.*) states that oxidation of the aldehyde and aldehydo-acid produced on ozonolysis of vaccenic acid (4 g.) led to the isolation of liquid monobasic acids (0·8 g.) (Found : *M*, 135. Calc. for $C_7H_{14}O_2$: 130) and solid dibasic acids (2·6 g.) (Found : *M*, 215·8. Calc. for $C_{11}H_{20}O_4$: 216), m. p. 109·8°, and, mixed with authentic nonanedicarboxylic acid, m. p. 110—110·5°. We have often repeated this oxidation (employing potassium permanganate in acetone solution as oxidising agent) and have invariably found the dicarboxylic acid products of oxidation

---

* Geneva numbering.

to be a difficultly separable mixture of indefinite melting point, from which in only one instance (a specimen of vaccenic acid from cow-milk fat) were we able eventually to isolate a small quantity of nonanedicarboxylic acid. Again, on effecting partial separation of the monocarboxylic acid products of oxidation by distillation through a small fractionating column we have observed consistent evidence of the presence therein of both *n*-heptanoic and *n*-octanoic acids; the range of boiling point and the equivalents of the distilled acids stand in marked contrast to those observed when the monobasic acids produced by the oxidative scission of oleic or elaidic acids (cf. Experimental, p. 3489) are distilled.

Bertram assigned a *trans*-configuration to the acid partly on account of its relatively high melting point but also because he was unable to convert it by the usual isomerising agents into a higher-melting or " elaidic " form. We have on several occasions submitted specimens of vaccenic acid to the action of selenium at 220°, and have found the product to consist mainly of the original acid, accompanied by smaller proportions of a liquid isomeric acid. This of course confirms that the natural acid is a *trans*- or " elaidic " isomer, the action of selenium resulting in the production of the equilibrium mixture of *trans*- and *cis*-forms in which (in the higher monoethenoid acids) the former predominates (cf. Griffiths and Hilditch, *J.*, 1932, 2315).

The dihydroxystearic acids produced when vaccenic acid is oxidised (i) in alkaline media and (ii) with peracetic acid conform in melting point with those which would be expected to result from the hydroxylation of a *trans*-ethenoid acid. A specimen of vaccenic acid isolated from cow butter-fat yielded, on oxidation with dilute ice-cold alkaline permanganate solution (Lapworth and Mottram, *J.*, 1925, **127**, 1628) a dihydroxystearic acid which melted at 94·5°; when peracetic acid (Scanlan and Swern, *J. Amer. Chem. Soc.*, 1940, **62**, 2305) was employed as oxidising agent the product was a dihydroxystearic acid which finally melted at 126°. More crystallisations than usual were necessary in each instance before the melting point reached approximate constancy; this again points to the probability that the vaccenic acid in question was a mixture of more than one of the higher *trans*-ethenoid fatty acids, with consequent formation of a mixture of dihydroxystearic acids of similar stereochemical configuration.

Since elæostearic esters from tung oil have been stated (Groot *et al.*, *loc. cit.*) to yield vaccenic acid (*trans*-octadec-11-decenoic acid) on partial hydrogenation, the nature of the octadecenoate esters produced by this means has been further investigated, and we have confirmed the view expressed by Hilditch and Pathak (*Proc. Roy. Soc.*, 1949, *A*, **198**, 323) that the chief primary products (monoethenoid esters) of hydrogenation are not homogeneous, but contain other monoethenoid isomers of the *trans*-octadec-11-enoate (which, however, is the predominant component).

The new factor which has emerged from the studies of the American workers, taken in conjunction with the facts now presented, is that natural " vaccenic " acid is undoubtedly a mixture of more than one *trans*-ethenoid acid, and certainly contains *trans*-octadec-10-enoic as well as *trans*-octadec-11-enoic acid. This puts a completely new aspect on the matter: when Bertram published his original data, it appeared that vaccenic acid was a unique instance of the production of a *trans*-higher monoethenoid acid in Nature. With the evidence now put forward for the presence of at least two isomeric *trans*-acids in " vaccenic " acid it seems more likely that this is not the complete picture. There is no proof that corresponding *cis*-forms of these trace acids are not also present in the animal fats concerned, for such forms would have passed, during the preliminary separation of the vaccenic acid as insoluble lead salt, into the more soluble portions of the fatty acid lead salts, and would have escaped detection. On the other hand, the presence of traces of isomeric forms of oleic acids in many animal fats and a few vegetable fats has long been recognised. Milliken and Brown (*J. Biol. Chem.*, 1944, **154**, 437) found that, whilst the octadecenoic acid in chicken fat, groundnut, cottonseed, maize, and linseed oils was wholly oleic acid, yet in some fats of animal origin (such as pig and ox depot fats, adrenal phosphatides, pig liver fats, and human body fat), and to a lesser extent in soya bean oil and rape seed oil, small quantities of an isomeric octadecenoic acid accompanied ordinary oleic acid (which formed almost the whole of the octadecenoic acids present). Moore (*J. Soc. Chem. Ind.*, 1919, **38**, 320T) recorded the presence in Antarctic whale oil of an isomer of oleic acid, which gave rise to a dihydroxystearic acid, m. p. 124—125°. Armstrong and Hilditch (*J. Soc. Chem. Ind.*, 1925, **44**, 180T) obtained a concentrate of an isomer of oleic acid (but still accompanied by the latter) from the least soluble lead salts of whale oil fatty acids: from the products of its disruptive oxidation they isolated *n*-heptanoic acid and small amounts of a dicarboxylic acid (equivalent 103·5) which suggested the presence of an octadec-11-enoic acid. A dihydroxystearic acid, m. p. 125—126°, was also obtained. These results are thus very similar to those obtained with natural vaccenic acid.

Taking all these facts into consideration, it may well be that the traces of *trans*-monoethenoid acids found in some animal fats, notably those of ruminants, are the result of secondary changes effected in the natural *cis*-glycerides synthesised by the animal or derived from its diet, and leading to equilibrium mixture of *cis*- and *trans*-forms of isomeric octadecenoic acids (the *trans*-isomers alone would be isolated by the techniques employed to concentrate the less soluble isomers of ordinary oleic acid). On this hypothesis, the mixture of *trans*-acids termed " vaccenic " acid merely represents part of the by-products or debris resulting from secondary changes of a minor order and is not a primary constituent of the natural body or milk fats in question ; in which case, particularly having regard to the need for much more information on the composition of the natural fats produced in a much wider variety of animals than has yet been studied, it may be unfortunate that a disproportionate amount of effort has been expended on the study of these trace components of a relatively small group of animal fats—an amount incommensurate with their probably insignificant intrinsic importance.

EXPERIMENTAL.

The data recorded below refer to studies of vaccenic acid from different sources carried out by one or other of the co-authors of this paper (as indicated in the side-headings).

(*a*) *From Ox Depot Fat.* (1938, S. PAUL.)—Solid fatty acids accumulated in the course of a study of the component glycerides of a perinephric fat from a 3-year old Shorthorn (Hilditch and Paul, *Biochem. J.*, 1938, **32**, 1775) served as the source of vaccenic acid. The acids (460 g.) were melted and mixed with a solution of mercuric acetate (390 g.) dissolved in a mixture of glacial acetic acid (300 g.) and methyl alcohol (400 ml.), as recommended by Bertram (*loc. cit.*). The saturated fatty acid salts which separated after the solution had remained at room temperature for 24 hours were removed by filtration and pressing (without washing), and the unsaturated portion of the original acids (45 g. ; I.V., 49) was recovered from the filtrates. These acids were submitted to a lead salt separation from alcohol, the deposited solid acids (28·4 g.) being re-submitted to the mercuric acetate separation, whereupon 15·6 g. of unsaturated acids (I.V., 79·3) were recovered from the soluble mercuric salts. This material [with a further 5·4 g. of acids (I.V., 75) obtained by re-extracting all the insoluble mercuric salts with the acetic acid–methyl alcohol mixture] was employed in the subsequent experiments, since the only extraneous matter was almost certainly saturated acids.

Oxidation with potassium permanganate in acetone. The vaccenic acid concentrate (6·5 g.; I.V., 79) was converted into methyl ester (6·6 g.), which was dissolved in acetone (130 ml.) and oxidised with powdered potassium permanganate (66 g.). After the reaction products had been worked up in the usual manner, the monobasic acid products were separated by distillation in steam, and converted into ethyl esters which, on distillation at 15 mm. pressure, gave a main fraction with an equivalent of 159 (ethyl heptanoate, 158). The dicarboxylic acid products of oxidation were isolated and crystallised repeatedly from a number of different solvents (methyl alcohol, chloroform, water) but the highest m. p. attained was 96—98° (indefinite).

Oxidation with peracetic acid. The crude vaccenic acid (2·2 g.) was dissolved in glacial acetic acid (15 g.) and oxidised (Hilditch, *J.*, 1926, 1828) with perhydrol (3·5 g.). The recovered dihydroxystearic acid, after several crystallisations from ethyl acetate, melted at 126°.

Isomerisation with selenium. Vaccenic acid (4·0 g.) was heated at 200° for 3 hours with powdered selenium (0·01 g.), after which the product was separated into " solid " (2·98 g.) and " liquid " (0·98 g.) acids by a lead salt separation from alcohol. On oxidation with peracetic acid the " solid " acids gave the above dihydroxystearic acid (m. p. and mixed m. p. 126°), but the " liquid " acids similarly yielded a dihydroxystearic acid, m. p. 116° (raised to 122° on admixture with the acid of m. p. 126°). The " liquid " acids thus evidently still contained some of the original vaccenic acid in addition to a low-melting isomer. The production of the latter only in relatively small proportions afford chemical evidence that the original vaccenic acid possessed the *trans*-ethenoid configuration.

(*b*) *From Cow Milk Fat.* (1947, R. K. SHRIVASTAVA.)—Accumulated " solid " acids obtained during lead salt separations of butter fatty acids at different times in our laboratory were first crystallised systematically from ether at temperatures ranging from 0° to −50°. By this means the following separation was finally effected from 1691 g. of " solid " butter acids whose iodine values ranged from 8·0 to 17·4 :

Fractions of acids obtained by crystallisation.

Fraction.	G.	Iodine value.	Fraction.	G.	Iodine value.
A	990·2	2·9—4·9	*D*	123·0	19·0
B	98·4	10·0	*E*	96·6	47·4
C	249·4	13·6	*F*	120·9	52·4
				1678·5	

The acids *C, D, E*, and *F* were separately converted into methyl esters, which were distilled through an electrically-heated and packed fractionating column in a vacuum; ester-fractions with equivalents approximating to 290 were combined and hydrolysed, and the resulting acids again submitted to crystallisation from ether or acetone at low temperatures. There was thus obtained a series of acid fractions of sparing solubility with iodine values ranging from 10 to 70, with more soluble acid fractions of iodine value 80—95; the latter were rejected as being likely still to contain ordinary oleic acid. The less soluble acid fractions were united and submitted to the mercuric acetate separation (Bertram, *loc. cit.*) in order to free the unsaturated acid as far as possible from saturated acids. In all, there were thus finally obtained 36·6 g. of crude vaccenic acid, which was once more crystallised from acetone at −40°, whereupon 6·5 g. of acids (I.V., 80·7) were left in solution and rejected as possibly containing oleic acid, whilst 30·1 g. of acids solid at room temperature (I.V., 74·0) were deposited. These acids, although still not free from saturated acids, were probably no longer contaminated with oleic acid and were employed in the following experiments.

Oxidation with potassium permanganate in acetone. The methyl esters (14·4 g.) from the vaccenic acid (I.V., 74·0) were dissolved in acetone (144 ml.) and oxidised with powdered permanganate (72 g.), whereby (in addition to 3·0 g. of unoxidised, mainly saturated esters, of I.V. 3·0) there were produced 4·3 g. of monocarboxylic and 7·6 g. of dicarboxylic acids. The monocarboxylic acids gave the following results on fractional distillation at atmospheric pressure :

Wt., g.	0·52	0·30	1·33	0·58
B. p.	160—172°	172—182°	182—204°	204—214°
Equiv.	108·4	115·9	125·6	148·1

The equivalents indicate that heptanoic (130) and octanoic (144) acids were the chief components of the mixture, with smaller proportions of an acid or acids of lower molecular weight.

Attempts to isolate an individual acid or acids from the dicarboxylic acid products of oxidation were unsuccessful.

Oxidation with dilute alkaline permanganate (Lapworth and Mottram, *loc. cit.*). The vaccenic acid (1·3 g.) was converted into potassium salt, and the latter oxidised at 0° in solution in water (1150 ml.) in presence of excess of potassium hydroxide (1·0 g.) with potassium permanganate (1·5 g.). After removal of saturated acids from the isolated products of oxidation by repeated washing with cold light petroleum, the crude dihydroxystearic acids (0·77 g.) were repeatedly crystallised from ethyl acetate and finally from methyl alcohol, from which ultimately a small amount of material which melted sharply at 94·5° was obtained.

Oxidation with peracetic acid. The crude vaccenic acid (1·34 g.) was oxidised by the procedure of Scanlan and Swern (*loc. cit.*). The acids recovered after refluxing the products obtained with alcoholic potassium hydroxide were crystallised from light petroleum (to remove saturated acids), and then from ethyl acetate. After recrystallisation from this solvent 0·3 g. of dihydroxystearic acid, m. p. 126°, was produced.

Isomerisation with selenium. Crude vaccenic acid (2·3 g.) was heated at 220° in an inert atmosphere for an hour in presence of powdered selenium (0·02 g.). The product (which had approximately the same m. p., 34—35°, as the original material) was crystallised from acetone at −40°; 1·35 g. of acids (I.V., 71·1) were deposited, leaving 0·55 g. (I.V., 80·9) in solution. The soluble acids (0·4 g.) were oxidised by dilute ice-cold alkaline permanganate, and a dihydroxystearic acid, m. p. 127° was produced.

(*c*) *From Sheep Depot Fat.* (1949, S. S. GUPTA.)—Solid fatty acids accumulated in the course of earlier studies of sheep body fats (Hilditch and Pedelty, *Biochem. J.*, 1941, **35**, 932; Hilditch and Zaky, *ibid.*, p. 940) were systematically crystallised from 10% solutions in ether at the temperatures shown in Fig. 1 (iodine values of each fraction are given in parentheses).

The fractions *D* and *E*, although probably rich in vaccenic acid, were not used in the subsequent constitutive experiments since they may still have contained small quantities of ordinary oleic acid. It was desired, in this section of the work, to study still more closely the mono- and di-basic products of oxidation of the solid isomers of oleic acid from the sheep fat, and for this purpose the fractions *A*, *B*, and C were utilised. The presence of large amounts of saturated acids does not interfere, since the corresponding saturated esters remain as unchanged neutral material after the oxidation.

A further quantity of the original " solid " acids (189 g.) was later submitted to the same series of crystallisations from ether, whereby, in addition to very sparingly soluble material (*A′*, 94 g., I.V., 2·0; and *B′*, 69 g., I.V., 3·1) there was obtained a fraction *C′* (8·7 g.; I.V., 41·4), the esters of which were used for ozonolysis.

Oxidation with potassium permanganate in acetone. (i) The methyl esters of fractions *A* and *B*, which contained only about 3% of unsaturated material, were nevertheless examined because the latter certainly consisted entirely of solid isomers of oleic acid. The esters (215 g.) were oxidised in acetone (1000 ml.) with powdered potassium permanganate (25 g.), which left 211·5 g. of unattacked esters (I.V., 1·9) and gave 0·35 g. of monobasic acids (an amount too small for further examination) and 2·16 g. of crude dibasic acids. The latter, after being refluxed with light petroleum to remove further traces of monobasic acids, were crystallised from water and then (0·5 g.) melted at 123—126°. Further crystallisation from water raised the m. p. to 130—131·5° (mixed with sebacic acid, $[CH_2]_8(CO_2H)_2$, m. p. 130—132°). The light petroleum solution deposited solid acids which, after several crystallisations from water, still melted indefinitely at 90—100°.

(ii) The methyl esters of fraction *C* (14·8 g.; I.V. of acids, 48·2) were similarly oxidised in acetone (300 ml.) with permanganate (30 g.), leaving 7·1 g. of unattacked esters (I.V., 3·0) and giving 2·9 g. of crude monobasic and 4·4 g. of crude dibasic acids. The monobasic acids were fractionally distilled at 15 mm. pressure with the following result :

Wt., g.	0·35	0·54	0·57	0·67	0·52
B. p.	110—120°	120—125°	125—127°	127—130°	130°
Equiv.	107·0	130·0	135·9	141·0	141·9

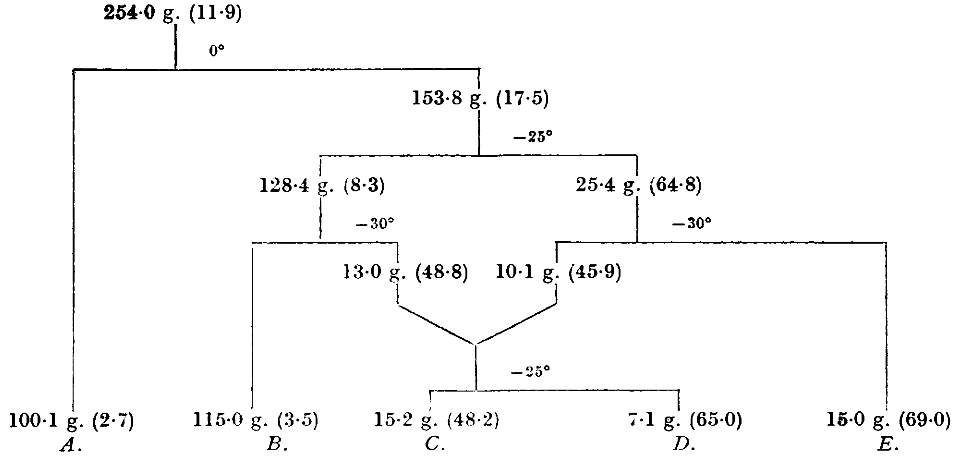
The dibasic acids (4·3 g.) were separated in chloroform at 0° into an insoluble (2·0 g.; m. p. 120—125°) and a soluble (2·3 g.; m. p. 90—115°) fraction. The insoluble fraction, on further crystallisation from ether and then from water, deposited 1·2 g. (m. p. 129—132°) which on further crystallisation from water gave 0·8 g. (m. p. 131—132·5°; equiv., 101·6). Admixture with sebacic acid (equiv., 101·0) caused no alteration in the m. p.

The acids soluble in chloroform were separated by ether at 0° into 0·7 g. of insoluble acids, m. p. 80—100°, which after further crystallisations from water yielded crystals of m. p. 120—125°; the soluble fraction (equiv., 109·5) was further separated by ether at —30° into an insoluble and a soluble fraction which, after further purification from water, respectively melted indefinitely at 105—113° and 90—100°.

This experiment therefore clearly indicated the presence of both heptanoic (equiv., 130) and octanoic (equiv., 144) acids in the monobasic products, whilst octanedicarboxylic (sebacic) acid was definitely isolated in quantity from the dibasic products; the latter also evidently contained more soluble acids which gave indications of containing nonanedicarboxylic acid (equiv. 108; m. p. 111—112°). The presence of octadec-10-enoic acid in considerable quantity, and its probable accompaniment by octadec-11-enoic acid, are thus definitely established in the " vaccenic " acid of the sheep body fat.

FIG. 1.

Crystallisation of " solid " acids from sheep body fat.



Oxidation by ozone. The methyl esters (8·7 g.) of fraction *C′* (I.V., 41·4) were dissolved in methyl acetate (43·5 ml.), and a current of ozonised oxygen passed through the solution at about —60° for an hour. The solvent was removed in a current of dry air and the ozonide decomposed by refluxing it with water; hydrogen peroxide solution was then added and the mixture left for 3 days. On working up of the products 5·6 g. of neutral esters (I.V., 12·3), 0·3 g. of monobasic acids, and 2·3 g. of crude dibasic acids were obtained. The quantity of monobasic acids was insufficient for further examination, but the dibasic acids, when crystallised from chloroform at 0°, gave insoluble material which after recrystallisation from chloroform melted at 129—130° (mixed with sebacic acid, m. p. 131—132°). The acids soluble in chloroform were refluxed with light petroleum, which left a solid of indefinite m. p. (80—100°); further crystallisations from water led to the isolation of a small proportion of acid, m. p. 120—125°, but the accompanying acid of apparently lower m. p. could not be isolated in sufficient purity for its identity to be established.

(*d*) *From Cow Milk Fat.* (1949, S. S. GUPTA.)—The " solid " acids of butter-fat, separated as alcohol-insoluble lead salts [after prior removal of acids (mainly butyric and hexanoic) volatile in steam], contain considerable proportions of decanoic, lauric, myristic, and palmitic acids in addition to acids of the C_{18} series. Methyl esters (180 g.; I.V., 9·6) of " solid " butter-fat acids, accumulated in the laboratory from earlier work, were therefore first fractionally distilled in a vacuum. When rather more than half of the esters had distilled over between 100° and 122°, the column-head temperature rose sharply to 128°, and the iodine value of the distilled esters (which up to that point had not exceeded 1·1) rose similarly suddenly to 7·0. The distillation was therefore terminated, and the residual undistilled esters were crystallised from 10% solution in ether at —35° in the manner shown in Fig. 2.

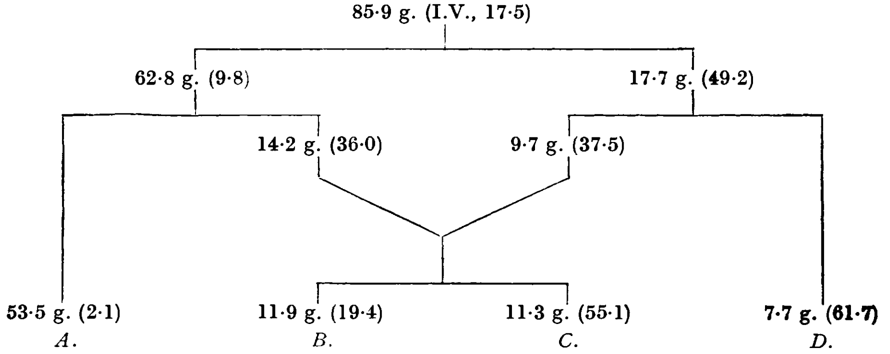
Fraction *D* was rejected in case of contamination with ordinary oleic ester.

Fraction *C* (11·0 g.; I.V., 55·1) was oxidised in acetone solution (220 ml.) with permanganate (30 g.) and yielded neutral products (4·0 g.; I.V., 5·9), monobasic acids (0·9 g., too small for further examination), and dibasic acids (2·6 g.). The crude dibasic acids were crystallised as in the case of those from sheep body fat vaccenic acid, and yielded finally a product which melted indefinitely at 100—105° (mixed with azelaic acid, m. p. 104°, depressed to 90—95°; mixed with nonanedicarboxylic acid, m. p. 110°, raised to 105—110°). This product, when left in acetone for three days at 0°, deposited a few crystals which melted at 108—110° (mixed with nonanedicarboxylic acid, m. p. 109—110°).

Thus, in this specimen of cow milk fat vaccenic acid, definite evidence was obtained of the presence of some octadec-11-enoic acid; but the behaviour of the dicarboxylic acid products of oxidation showed that another dibasic acid or acids (apparently not including azelaic acid) accompanied the nonanedicarboxylic acid. In the previous experiment (*b*) on cow milk fat vaccenic acid, in which sufficient monobasic acids were recovered to be distilled, it will be noticed that the greater part of the latter approximated in equivalent to heptanoic acid, with only a small proportion of acids with an equivalent close to that of octanoic acid. Both studies thus suggest the presence of more octadec-11-enoic than octadec-10-enoic acid in the cow milk fat vaccenic acid, whereas in the vaccenic acids of ox and sheep body fats [Experiments (*a*) and (*c*)] the proportions of the two isomeric octadecenoic acids appear to be reversed.

FIG. 2.

Crystallisation of methyl esters of " solid " acids from cow milk fat.



(*e*) *Oxidation of Methyl Oleate and Methyl Elaidate with Potassium Permanganate in Acetone.* (1949 S. S. GUPTA.)—A study of the disruptive oxidation of methyl oleate and methyl elaidate was undertaken (i) to obtain comparative data regarding the composition of the monobasic acidic products of oxidation and (ii) to satisfy ourselves that esters of *trans*-acids behave similarly to those of *cis*-acids under the conditions of oxidation (a point which appeared not to have been definitely established in previous work).

(i) *Methyl oleate.* Methyl oleate was prepared from carefully purified oleic acid (Hilditch and Pathak, *loc. cit.*) which was shown by spectrophotometric analysis to contain no linoleic acid, but which contained about 2% of stearic acid. The ester (10·1 g.; I.V., 84·0) was treated in acetone solution (202 ml.) with permanganate (40·4 g.); the products of oxidation were worked up as usual, and there were obtained neutral (unoxidised) esters (0·7 g.), crude monobasic acids (4·1 g.), and crude dibasic acids (4·5 g.). Two crystallisations of the last-named from water (after preliminary removal of any residual traces of monobasic acids with light petroleum) gave 2·7 g. of crystalline acid (equiv. 95·2; m. p. 103—103·5°, unchanged on admixture with azelaic acid). The monobasic acids, when distilled at 15 mm. pressure through a small fractionating column, gave the following fractions:

Wt., g.	0·88	1·20	1·20
B. p.	140—148°	148—150°	150—152°
Equiv.	149·2	151·8	153·9

(ii) *Methyl elaidate.* Elaidic acid was prepared by heating purified oleic acid (54·6 g.) with powdered selenium (0·1 g.) at 190—200° for two hours in a current of nitrogen. The product, when crystallised successively from ether at —15°, —25°, and —30°, deposited the following crops of elaidic acid: 21·0 g., m. p. 44°; 7·1 g., m. p. 43—43·5°; and 10·4 g., m. p. 41—42°. The total yield of pure elaidic acid was thus somewhat less than 38 g., corresponding to about 70% of elaidic acid in the equilibrium mixture of *cis*- and *trans*-acids produced (cf. Griffiths and Hilditch, *loc. cit.*).

Methyl elaidate was prepared from the purest fraction of the elaidic acid (m. p. 44°), and a quantity (10·0 g.) was oxidised in acetone (200 ml.) with permanganate (40·0 g.), neutral (unoxidised) esters (0·3 g.), crude monobasic (4·8 g.), and crude dibasic (5·2 g.) acids being obtained. Two crystallisations of the dibasic acids from water gave 1·8 g. of pure azelaic acid (m. p. and mixed m. p. 105°), whilst a further crop of acid (1·5 g., m. p. 100—102°) was recovered from the mother-liquor. The disruptive oxidation of the *trans*-ester thus took place even more smoothly and efficiently that that of the *cis*-ester. The monobasic acids, distilled at 10 mm. pressure, gave the following fractions:

Wt., g.	1·17	1·23	0·90
B. p.	115—120°	120—140°	140—150°
Equiv.	147·0	150·5	152·5

The monocarboxylic acid fractions from the oxidation of either methyl oleate or methyl elaidate do not reach the equivalent of nonanoic acid (158), but on the other hand the equivalents of the lower-boiling fractions are only about 5 units below the maximum values reached. The presence of some proportion of acid of lower molecular weight than nonanoic must be attributed to secondary oxidation of the monocarboxylic acid produced in the initial oxidation (cf. Armstrong and Hilditch, *J. Soc. Chem. Ind.*, 1925, **44**, 43T). Nevertheless, the fractions of monocarboxylic acids obtained in the oxidation of methyl oleate and elaidate stand in marked contrast to the corresponding fractions from the oxidation of the methyl esters of vaccenic acid from cow milk fat (*b*) or sheep depot fat (*c*). In the latter, the total range in equivalents of the acid fractions is from about 108 to over 140; when due allowance is made for the effects of secondary oxidation (as shown above), it remains clear that mixtures of at least two primary monocarboxylic products of oxidation resulted from the vaccenic esters.

(*f*) *Oxidation of Hydrogenated Methyl Elæostearate from Hydrogenated Tung Oil.* (1949, S. S. GUPTA.) —Whereas Boeseken *et al.* (*loc. cit.*) stated that octadec-11-enoic acid was selectively produced at one stage of the hydrogenation of elæostearic esters, and Groot *et al.* (*loc. cit.*) recommended this procedure as a means of production of vaccenic acid, Hilditch and Pathak (*loc. cit.*) isolated from methyl elæostearate which had been hydrogenated to a point (iodine value 75) at which nearly all elæostearate had disappeared (but which then contained some stearic acid) about 35—40% of a solid monoethenoid acid which melted at 39—40°. This acid appeared to consist largely of *trans*-octadec-11-enoic acid, but the presence of other isomeric acids was also indicated. We have therefore re-examined this point. For this purpose we selected a specimen of tung oil which had been hydrogenated further than in the previous study, in order to avoid as far as possible the presence of polyethenoid products of hydrogenation.

The acids (140 g.) from a specimen of hydrogenated tung oil (I.V., 47) were first crystallised from ether successively at 0° and at −20°, concentrates of saturated acids (50 g., I.V., 9·5; and 26 g., I.V., 28·5) being removed. The remaining acids (64 g., I.V., 93·0) were further crystallised from ether at temperatures down to −50°, and eventually a fraction of 20·5 g. (I.V., 81·6) was obtained which melted at 38·5—40·5° (mixed with natural " vaccenic " acid from butter-fat, m. p. 36—38°). The rest of the monoethenoid acids (I.V., 95—98) were still contaminated with small proportions of diethenoid acids.

It may be remarked that the m. p. of these " *iso*oleic " acids appears to be of little use as a guide to their identity. Elaidic acid itself melts at 44°, specimens of natural " vaccenic " acids are also recorded by different workers as melting at temperatures between 40° and 43°, and the *iso*oleic acids produced by partial hydrogenation of oleic or linoleic esters also melt within this range (cf. Moore, *loc. cit.*). Moreover, mixtures of these various acids appear as a rule to melt not more than 4° or 5° below the m. p.s of the individual acids.

The hydrogenated acids of I.V. 81·6 were converted into methyl esters, of which a portion (11·5 g.) was oxidised in acetone (230 ml.) with permanganate (46 g.). There resulted neutral (unoxidised) esters (0·8 g.; I.V., 21·7), monobasic (3·8 g.), and dibasic (7·0 g.) acids. The monobasic acids gave the following fractions on distillation in a vacuum (0·2 mm.):

Wt., g.	0·80	1·26	1·32	0·41
B. p.	60—80°	80—100°	100—110°	Residue
Equiv.	111·4	123·1	138·5	165·6

The acids were clearly a complex mixture with heptanoic acid (equiv., 130) predominating, but also including octanoic, hexanoic, and probably nonanoic acids.

Crystallisation of the crude dibasic acids led to no definite result, and they were therefore recovered, recombined, and converted into dimethyl esters which were also fractionally distilled in a vacuum through a small column. The results were as follows:

Wt., g.	0·54	0·79	0·56	0·70	0·73	0·85
B. p.	95—110°	110—120°	120—122°	120—122°	120—122°	122—125°
Equiv.	111·5	113·0	114·5	122·2	124·3	132·4

The equivalents of the dimethyl esters of azelaic, sebacic and nonanedicarboxylic acids are respectively 108, 115, and 122. It therefore appears that nonanedicarboxylic acid was the chief dibasic acid constitutent, and that sebacic acid was also present in some quantity, whilst smaller amounts of a lower acid (presumably azelaic) and of a higher acid (? dodecanedicarboxylic acid) were also present. The acids from the fraction of equivalent 124·3 were recovered and crystallised from water several times, about 0·1 g. of an acid of m. p. 102—105° (mixed with nonanedicarboxylic acid, m. p. 100—106°) being obtained.

The evidence from both the mono- and di-basic acids from the oxidation thus points to a mixture of several octadecenoic acids having been present. From both analyses it would appear that octadec-11-enoic acid is the most abundant isomer, since heptanoic acid and nonanedicarboxylic acid are the largest components in each group of acids; but there is also evidence for the presence of other octadecenoic acids in lesser amounts, including the Δ^{10}- and probably also the Δ^{9}- and Δ^{12}-acids.

The complex nature of the monoethenoid acids was also indicated by the formation, either by the action of aqueous alkaline permanganate or of peracetic acid, of a mixture of dihydroxystearic acids from which no individual component could be isolated. On the other hand, more drastic oxidation with aqueous alkaline permanganate (Lapworth and Mottram, *J.*, 1925, **127**, 1987; Green and Hilditch, *ibid.*, 1937, 764) led to the formation of mono- and di-basic acids, and from the latter a small proportion of an acid (m. p. 131—132°, unchanged when mixed with sebacic acid) was finally isolated. This is further evidence that octadec-11-enoic acid was one of the chief constituents of the mixture of monoethenoid acids produced during the hydrogenation of elæostearic glycerides.

We thank Messrs. Lever Brothers & Unilever Ltd. for a Lever Research studentship held by one of us (S. P.), and the Government of India for scholarships (S. S. G., R. K. S.) held at the periods in which this work was in progress.

THE UNIVERSITY, LIVERPOOL.

[*Received, August* **22*nd***, 1950.]