Trans-Octadecenoic Acid Content of Beef Fat. Isolation of Elaidic Acid From Oleo Oil[1,2]

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As part of a systematic investigation of the infrared spectrophotometric characteristics of fats, purified fatty materials and oxygen-containing derivatives (13, 14, 15, 16, 19), we examined five samples of edible beef fat obtained from steers slaughtered at widely different times. One sample was a freshly prepared commercial oleo stock and consisted of a composite of the fat obtained from all the tissues normally used in such preparations. Two samples consisted of commercial oleo oil and oleo stearine obtained from this oleo stock in the conventional way. The other two samples were the total fats isolated under carefully controlled conditions in our laboratory from fresh fatty tissue obtained from the pleural cavity and kidney region. Infrared analysis (13, 19) of the oleo stock, the pleural and kidney fat indicated a trans-acid content (calculated as trielaidin) of about 5-8% whereas the oleo oil contained about 6% and the oleo stearine about 10% of trans-acids, respectively. Since we had shown in an earlier publication (11) that the non-conjugated dienoic and trienoic acids of beef fat are mainly, if not exclusively, cis-acids, it follows that the trans-acids of beef fat are mainly, if not exclusively, monounsaturated.

The content of trans-monounsaturated acids in beef and certain other animal depot fats has usually been assumed to be in the range of 0.1% to a maximum of about 2% (2, 5, 6). These values have been obtained by calculation from the iodine number and weight of solid fatty acid fractions isolated from the mixed fatty acids of a fat by lead salt precipitation. Admittedly such values are low since it is now well-known that the lead salt technique does not cause trans-monounsaturated acids to precipitate quantitatively, and, in fact, the error may be exceedingly large (7, 19). The infrared spectrophotometric method (7, 13, 19) for determination of trans-octadecenoic acids however is known to be reliable. The assumption therefore that trans-monounsaturated acids occur only in traces in beef fat is no longer tenable. Furthermore the content of trans-monounsaturated acids in edible beef fat exceeds the content of polyunsaturated acids, and it must be concluded that the trans-acids are not minor nor adventitious constituents but important naturally-occurring components which may contribute to any unique properties which beef fat may have. Since beef fat contains a total of about 40-50% of monounsaturated acids, the trans-acids comprise about 10-15% of the monounsaturated acids. This significant percentage of trans-acids would account for the low melting-points of octadecenoic acids isolated from certain animal fats, notably beef fat and lard, and the corresponding dihydroxystearic acid prepared from them (12).

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Isolation of Elaidic and Vaccenic Acids

Vaccenic acid was required as a reference compound for several studies in progress in our laboratory. Oleo oil was selected as a suitable starting material since vaccenic acid had been obtained from it (2).

Examination of the published procedures prompted us to try to improve and simplify the isolation technique. The main variations introduced were, first, precipitation of the bulk of the saturated acids from the mixed acids of oleo oil by low temperature crystallization from acetone at —20°; second, preparation of a polyunsaturate-free C-18 fraction by crystallization of the octadecenoic acids from acetone at —80° prior to the lead salt separation; third, elimination of the mercuric acetate treatment. The early elimination of the bulk of the saturated acids by solvent crystallization permits the handling of much smaller quantities of lead salts later in the procedure. The isolation of a polyunsaturate-free fraction prior to a lead salt separation was considered desirable in view of the activity of lead salts of polyunsaturated acids in catalyzing autoxidation.

It was recognized that lead salts of polyunsaturated acids are readily soluble in alcohol and would therefore be unlikely to precipitate, but even small quantities occluded in the main precipitate fraction might be sufficient. This was especially important since the lead salt separation was to be applied to an oleic acid-rich fraction in which lead oleate would be very likely to precipitate (19). It was believed that the mercuric acetate separation could be eliminated in view of the separaton of saturated acids effected by fractional crystallization earlier, and this would simplify the procedure substantially. The net effect of these modifications was indeed a simplified procedure, but it also resulted in the isolation of an elaidic acid-rich fraction in addition to one which was primarily vaccenic.

Experimental

Starting Materials. Oleo stock was a freshly prepared commercial specimen and consisted of a composite of the fat obtained from all the tissues normally used in such preparations. Oleo oil and oleo stearine were obtained from the oleo stock in the conventional commercial way. Kidney and pleural fat were prepared by mixing the cut-up fatty tissue from the kidney and pleural region, respectively, with hexane in a high-speed blendor; the operation was conducted under nitrogen. Filtration and evaporation of solvent yielded the fats as residues. Infrared analyses (13, 19) were conducted on carbon bisulfide solutions containing about 10 g. of fat per 100 ml. A Beckman IR-3 Infrared Spectrophotometer was employed.

Isolation of Elaidic Acid from Oleo Oil. Preparation of Polyunsaturate-Free C-18 Fraction. Oleo oil [14 kg.; iodine number 46.6; content of trans materials (13, 19) calculated as trielaidin, 5.6%] was saponified by the rapid technique previously described (18). The yield of mixed fatty acids, iodine number,

49.1, was quantitative. Twelve thousand three hundred g. of these were crystallized from acetone (6 ml. of solvent per gram of solute) first at 0° (precipitate, 4,200 g.) and then at —20° (precipitate, 1,600 g.) to remove the bulk of the saturated acids. The acetone was recovered from the filtrate, and the residual acids (6,200 g.) were rapidly distilled through a low pressure-drop column 15 inches by 2 inches, with a special packing (7-10 theoretical plates) (8). The C-18 fraction, b.p. 203-209° at 3.6-3.8 mm. and weighing 4,300 g. (iodine number, 94.6; content of trans materials calculated as octadecenoic acids, 13% or 559 g.), was crystallized at —80° from acetone (15 ml. of solvent per gram of solute). The polyunsaturate-free precipitate weighed 3,600 g. and was a pale yellow liquid (iodine number, 87.9; content of trans-octadecenoic acids, 11% or 396 g.).

Lead Salt Separation. Three thousand g. (operation conducted in three 1,000 g. batches) of the polyunsaturate-free C-18 fraction was dissolved in 15 liters of 95% ethanol. To this solution at the boiling point, a hot solution of 540 g. of lead acetate in 15 liters of 95% ethanol containing 450 ml. of glacial acetic acid was added. The solution was cooled to 0 to 5° overnight and rapidly filtered by suction. A positive test for lead was obtained in the filtrate. The precipitated lead salts were recrystallized at 0 to 5° from 95% ethanol containing sufficient glacial acetic acid (approximately 1.5% of the total volume) to yield a homogeneous solution. The recrystallized, dry lead salts were converted to free acids by gentle warming with an excess of 6N nitric acid, and the free fatty acids separated as an oily layer which solidified on cooling in the refrigerator. The aqueous acid layer was discarded, and the free fatty acids were washed until acid-free with successive portions of warm water followed by chilling and discarding the aqueous layer. The free fatty acids weighed about 530 g. (iodine number, 61; m.p., 36.8-37.4°).

Methyl Ester Fractionation. Since the original fractional distillation of the free acids did not separate palmitic acid completely, the acids were converted to methyl esters by refluxing for several hours with a large excess of methanol containing naphthalene-2-sulfonic acid as catalyst, and the resulting methyl esters were fractionally distilled through a packed column 36 inches by ¾ inch. The fractions boiling at 187-191° at 4.7 to 5.0 mm. and n_D^{30} 1.4461 to 1.4472, were combined. They weighed 250 g.; iodine number, 80.

Isolation of Concentrates of Trans-Acids. The methyl esters were converted to free acids (yield, 240 g.) and these were fractionally crystallized from acetone (10 ml. of solvent per gram of solute) to ensure complete separation of oleic acid which might still be present. The precipitate at 0° weighed 41 g. (iodine number, 80.1; m.p. 39.3-40.0°) and that at —20° weighed 93 g. (iodine number, 85; m.p. 37.2-38.5°, neutralization equivalent, 280). The —20° fraction was recrystallized, yielding a precipitate (E-1) at 0° which weighed 27 g. (iodine number, 82.3; m.p. 40.0-41.8°; neutralization equivalent, 282) and a precipitate (E-2) at —20° which weighed 45 g. (iodine number, 86.2; m.p. 37.0-37.5°, neutralization equivalent, 282).

Identification of Trans-Acid Fraction E-1. This was shown to contain elaidic acid as its main trans component. Hydroxylation of 7.1 g. of this material with hydrogen peroxide and formic acid (17) gave 5.1 g. (70% yield based on unsaturated acid content of E-1) of slightly impure 9-10-dihydroxystearic acid, m.p. 125°, after two crystallizations from 95% ethanol. A mixed melting point (1:1) with authentic 9,10-dihydroxystearic acid, m.p. 131°, gave no depression. The melting point of the mixture (127-128°) was sharp and between that of the two samples. A mixed melting point (1:1) of the 9,10-dihydroxystearic acid obtained from E-1 with a sample of 11,12-dihydroxystearic acid, m.p. 128.5-128.8° (supplied by F. M. Strong), was significantly depressed (mixed melting point 115.5-116.6°).

A mixed melting point (1:1) of Fraction E-1 with authentic elaidic acid (m.p. 44°) gave only a slight depression in melting point whereas a mixed melting point with vaccenic acid (m.p. 41-42°) from butter fat (supplied by Miss P. Haverkamp Begemann) showed a large depression (mixed m.p. 33.2-33.9°). A mixed melting point of the butter fat vaccenic acid with authentic elaidic acid also showed a substantial depression in melting point (mixed m.p. 36-38°) whereas the melting point of a mixture of trans-11-octadecenoic acid, m.p. 42.5-43.5° (supplied by F. M. Strong), and butter fat vaccenic acid showed no depression whatever.

A description of some unusual melting point and x-ray diffraction characteristics of mixtures of isomeric synthetic trans-octadecenoic acids and also of their corresponding dihydroxystearic acids, and the use of such data for identity purposes, will be given in a subsequent report.

Identification of Trans-Acid Fraction E-2. This appeared to be a typical vaccenic acid. Its trans-acid content by infrared analysis (13, 19) was 87% whereas calculation of the trans-acid content from its iodine number, assuming all unsaturation caused by trans-acids, was 96%. This discrepancy is still unresolved but may be caused by the presence of relatively high-melting cis-octadecenoic acids which would precipitate with the trans-acids. Mixed melting point (1:1) with the butter fat vaccenic acid and synthetic trans-11-octadecenoic acid showed little or no depression in melting point (mixed m.p. 37.6-39.0° and 36.4-37.8°, respectively). Since this fraction appeared to be similar to the vaccenic acid investigated by Hilditch and coworkers (5), it was not studied further.

Discussion

The origin of trans-octadecenoic acids in beef fat is unknown, but some data which we have obtained may bear on this point.

The trans-acid content of the samples of beef fat studied in this investigation were about 5-8% whereas oleo oil and oleo stearine fractions obtained from one of them (oleo stock, trans-acid content, 5-6%) were 5.6 and 10%, respectively. The sum of the content of trans-acids in these fractions exceeded the trans content in the starting material by a significant amount. Since the commercial graining and pressing of beef fat to prepare oleo oil and oleo stearine are not designed to eliminate oxygen and, in fact, conditions are conducive for oxidation, we concluded tentatively that the increase in trans-acid content was caused by an oxidatively induced cis-trans isomerization.

Evidence to support this conclusion was recently obtained as a result of our infrared spectrophotometric study of the autoxidation of methyl oleate (10), in which it was shown that a cis-trans isomerization

occurred even during the earliest stages of oxidation. These "in vitro" experiments certainly do not prove that trans-octadecenoic acids arise in beef fat as a result of oxidative isomerization of cis-acids, but it is known that unsaturated fatty acid oxidases are present in animal tissues (1). If these oxidases can induce oxidation reactions in the fatty tissue, then possible mechanisms for the formation of trans-octadecenoic acids are as follows:

a) Oxidative cis-trans isomerization, coupled with double bond migration during oxidation (3, 4) could result in the formation of trans-11-octadecenoic acid (so-called vaccenic acid) from the oleic acid moieties in beef fat. A logical consequence of this mechanism is that trans-isomers other than trans-11-octadecenoic acid should also be formed. Hilditch and coworkers (5) have recently provided evidence to show that trans-10-octadecenoic acid is also present in animal depot fats, in some cases in considerable quantity. Double bond migration toward the carboxyl group cannot be precluded, but trans-octadecenoic acids in which the double bond is closer to the carboxyl group than the 9 position have not yet been identified.

b) Oxidative cis-trans isomerization without double bond shift would result in the formation of trans-9-octadecenoic (elaidic) acid from oleic acid.

c) Oxidative cis-trans isomerization with or without double bond shift of other (unknown) cis-acids present in the fat would also result in the formation of isomeric trans-octadecenoic acids. If cis-octadecenoic acids other than oleic acid are present in the fat, a possibility pointed out by Hilditch and coworkers (5), then the trans-10- and trans-11-octadecenoic acids, which have been isolated from beef fat, may arise from them rather than from oleic acid.

Since cis-9-octadecenoic acid is the major octadecenoic acid present in beef fat, we believe that the most likely mechanisms for the formation of transoctadecenoic acids are a) and b) above. Consideration must also be given however to the possibility of forming cis- and trans-octadecenoic acids from stearic acid by the action of fatty acid dehydrogenases. In the absence of any direct evidence regarding the actual mechanisms of formation of trans-octadecenoic acids in beef fat, the explanations given must be considered highly tentative and certainly not all-inclusive.

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Summary

Infrared spectrophotometric examination of three samples of freshly-rendered edible beef fat, and of edible oleo oil and oleo stearine obtained from one of them, has revealed the presence of substantial quantities (5 to 10%) of trans materials believed to be mainly, if not exclusively, monounsaturated. It has been concluded that the trans components are neither minor nor adventitious constituents but important naturally-occurring components which may contribute to any unique properties which beef fat may have.

It is proposed that trans-octadecenoic acids arise in beef fat as a result of an oxidative cis-trans isomerization coupled with double bond shift, reactions now known to occur in oxidizing fat systems, or by a simple oxidative cis-trans isomerization without double bond shift of oleic or other isomeric cis-octadecenoic acids which may be present in beef fat.

Trans-9-octadecenoic (elaidic) and vaccenic acids have been isolated from oleo oil, the former acid apparently for the first time.

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