Identification of 11,15-Octadecadienoic Acid From Beef and Mutton Tallow[1]

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Abstract

By means of gas liquid chromatography and thin layer chromatography on Silica gel G/$AgNO_3$ we isolated some isolinoleic acids from beef and mutton tallow, which by their chromatographical behavior, IR analysis and by double bond determination by ozonolysis are mainly stereo-isomeric 11,15-octadecadienoic acids and in a smaller amount stereo-isomers of 10,15-octadecadienoic acids. 4-*Cis*- and 4-*trans*-heptenals, 2-*trans*,6-*cis*- and 2-*trans*,6-*trans*-nonadienals have been isolated from the DNPH mixture obtained from the volatile decomposition products of an oxidized synthetic mixture of stereo-isomeric 11,15-octadecadienoic acids.

Introduction

In a previous paper (1) we described the isolation of 2-*trans*,6-*trans*- and 2-*trans*,6-*cis*-nonadienals and of 4-*cis* and 4-*trans*-heptenals from beef and mutton tallows. These aldehydes are at least partly responsible for some typical odors, characterized as tallowy, cucumberlike and greenish, developed in reverted tallows.

We obtained typical reversion flavors from the fatty acid part, which was freed from the unsaponifiable part. Our attempts to provoke reversion flavors from the unsaponifiable part, were not successful. Therefore, we decided that the precursors for the aldehydes that were isolated must be sought among the fatty acids of these fats. In this paper the isolation of these precursors will be described.

Fatty Acid Composition of Different Tallows

Tallows originating from different tissues or from different geographical locations may have different fatty acid compositions. The composition depends on many factors, but either differences in nutrition of the animals or differing proportion of the various fatty tissues used are the main causes of variation.

Some analytical data on fatty acid compositions of two samples of beef tallow are given in Table I. Gas liquid chromatography (GLC) was applied, using polyethylene glycol adipate (5%) on Diatoport S as immobile phase at 180 C, column length 200 cm, column diameter 4 mm, and flame ionization detection. The total *trans* double bond content of these tallows was 5%, as measured by IR spectroscopy.

Some gas chromatographic fractions were collected by condensation in U-tubes cooled by liquid nitrogen. The presence of double bonds in these fractions was established by IR spectroscopy.

In Australian beef tallow we could also establish that the peak corresponding to 15:1 (or branched 16:0) contained methyl 9-oxononanoate, an oxidative cleavage product of fatty acids having a C_9 double bond. As we also established the presence of double bonds by means of IR spectrometry we supposed, that more peaks (such as that of 18:2) may contain more than one fatty acid, some of them with a most uncommon double bond or carbon-skeleton pattern.

The existence of isomeric linoleic acids in lamb caul fat was established previously by Weenink (2), who determined in an ozonolyzed 18:2 fraction the presence of 1.6% malonic, 8.2% succinic, 2.9% glutaric and 3.1% adipic acid. He concluded that a considerable amount (1.2%) of nonconjugatable dienoic fatty acids having double bonds separated by two or more methylene groups was present in this particular fat.

In butterfat at least three isomeric *cis,cis*-linoleic acids, namely the 11,15-, 10,15- and 9,15-octadecadienoic acids were isolated by de Jong and van der Wel (3). Two of these acids, the 10-*cis*,15-*cis*- and 11-*cis*,15-*cis*- served as precursor for 4-*cis*-heptenal, isolated from butterfat by Haverkamp Begemann and Koster (4).

Concerning groups of the chain length (C_9 and C_7) and the structure (having *cis*- or *trans*- double bonds in the 3-terminal position) of the four aldehydes isolated by us (1) we supposed that some nonconjugatable isolinoleic acids could serve as precursors for these aldehydes in the case of tallows also.

Isolation of Fatty Acid Fractions by Means of TLC

As fractional distillation or urea inclusion is not able to give sharply separated fractions of dienoic tallow fatty acids, it was decided to use preparative TLC on $SiO_2/AgNO_3$.

Beef tallows of Australian and Dutch origin were freed of unsaponifiable matter and the fatty acids (obtained after acidifying the soap) were reesterified with methanol-sulfuric acid.

The methyl esters were applied to a thin layer (0.3 mm) of Silica gel G HR (Macherey Nagel) impregnated with 30% of silver nitrate and eluted with a mixture of benzene-light petroleum (70:30), following the method of De Vries (5) (Fig. 1, columns 3 and 4 of spots).

Some model substances, such as the methyl esters of stearic, oleic (9-*cis*-octadecenoic), linoleic (9-*cis*, 12-*cis*-octadecadienoic) and linolenic (9-*cis*,12-*cis*,15-*cis*-octadecatrienoic) acids were also applied to the plate (column 1) whereas in the second column 9-*cis*-hexadecenoic acid methyl ester and in the fifth column an isomerized mixture of synthetic 11,15-octadecadienoic acids (all four possible isomers) were also spotted. The original methyl 11-*cis*,15-*cis*-octadecadienoate was first synthesized by Pabon and van Dorp (6). This acid was isomerized by selenium as catalyst into the (four possible) stereoisomers.

As we see, the saturated and traces of branched fatty acid methyl esters (Table I) of tallow are not π-complexed therefore they elute nearly with the front of the solvent mixture (Band I, Fig. 1). In band II we find methyl 11-octadecenoate (*trans* isomer).

In band III we could establish the presence of 9-*cis*-octadecenoate and that of 9-*cis*-hexadecenoate besides a low amount of 11-*trans*,15-*trans*-octadecadienoic acid methyl ester. The following isolated band IV had the same R_f value as the model fatty acid, containing *cis,trans* and *trans,cis* nonconjugatable

[1] Presented in part by G. Hoffmann at the 7th ISF Congress, Hamburg, October 1964.

TABLE I

Fatty Acid Methyl Ester Composition of Two Samples of Beef Tallow

Fatty acid	C_n	Australian GLC %	Australian TLC bands[a] Checked by GLC I	II	III	IV	V	VI	Australian Checked by IR c.a. % *trans* III	IV	Dutch GLC %	Dutch TLC bands[a] Checked by GLC I	II	III	IV	V	VI	Dutch Checked by IR c.a. % *trans* II	III	IV
$C_{14:0}$	14	4.5	M								3.5	M								
$C_{14:1}$	14.5	0.5	Br		S						1.5	Br		S						
	14.7		Br		Trace							Br		Trace						
$C_{15:0}$	15	0.5	S								1	S								
$C_{15:1}$	15.4	Trace	Br		Trace						0.5	Br		Trace						
	15.6																			
$C_{16:0}$	16	28.5	L								26.5	L								
$C_{16:1}$	16.4	2.5	Br		M						4	Br		M						
	16.7	0.5	Br		Trace						1	Br		Trace						
$C_{17:0}$	17	1	S								1.5	S								
$C_{17:1}$	17.4	Trace	Br		S						1	Br		S						
	17.5																			
$C_{18:0}$	18	25	L								21.5	L								
$C_{18:1}$	18.3	34		M	L						34		M	L				70		
$C_{18:2}$	18.7	1				L	L	2	8		1				L	L			1.5	10
	18.9										2									
$C_{19:0}$	19	1																		
$C_{19:3}$	19.3	Trace			Trace			M			Trace						M			
	19.7	0.5									0.5									
$C_{20:0}$	20	Trace	Trace								Trace									
$C_{20:1}$	20.3	0.5									Trace			Trace						
	20.5	Trace									Trace			Trace						
	20.7													Trace						
		99.5									99.5									

[a] Abbreviations; Br, presence of branched fatty acids; L, large; M, medium; S, small.

double bonds. IR spectrometry showed that this band contained about 10% of *trans*-unsaturation. Besides, the methyl esters present in this band must be methyl 9-*cis*,12-*cis*-octadecadienoate, as the R_f value of this ester corresponds to that of the above-mentioned isolinoleic methyl esters.

Band V had the same R_f value as the model *cis,cis* nonconjugatable isolinoleic acid methyl ester, whereas band VI (well separated) showed the R_f value of methyl linolenate.

Location of Double Bonds in Dienoic Fractions by Means of Ozonolysis

To obtain information about the nature of the fatty acids present, a technique of reductive ozonolysis, described by Beroza and Bierl (7) as modified by Han (private communication) was applied to some of the fractions. They were scraped off from the plate, extracted, redissolved in CS_2 and ozonolyzed in the cold (−20 C). The ozonides were immediately reduced in the cold by triphenylphosphine and the aldehydes (A), dialdehydes (DA) and aldehydic esters (AE) obtained directly injected on a temperature programmed (60–200 C) GLC apparatus with double flame ionization detection. The immobile phases were Diatoport S with 10% Carbowax 20 M and Gaschrom Q with 10% PEGA, the mobile phase nitrogen. Column length was 200 cm, column diameter 4 mm.

The results of the GLC analysis of the different fragments are given in Table II.

The aldehyde groups in the aldehydic esters represent the location of the first double bond counted from the carboxylic acid end of 18:2 present in the bands.

The dialdehydes are a measure of the length of carbon chain fragments connecting two double bonds of the chain. The carbon atoms with the aldehyde groups originate from the second C-atom of the first double bond and the first C-atoms of the second double bond.

The aldehydes represent the chain length of the end fragment of an ozonolyzed fatty acid, beginning with the second carbon atom of the last double bond in the chain and ending with the methyl group of the original fatty acid.

From these data we drew the following conclusions:

From the fragments in the well separated band II (Fig. 1) we could confirm the presence of the postulated methyl-11-*trans*-octadecenoate (C_{11} AE +

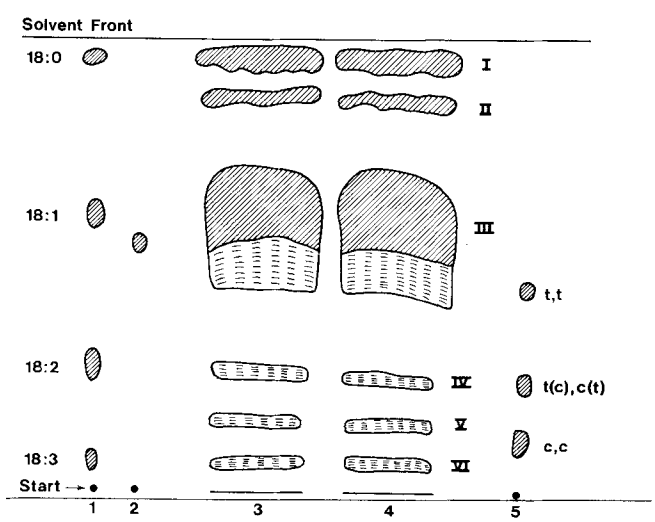

FIG. 1. TLC separation into fractions of methyl esters of Australian and Dutch beef tallow fatty acids: 1, mixture of common fatty acid methyl esters; 2, methyl ester of 9-*cis*-hexadecenoic acid; 3, methyl esters of Australian beef tallow fatty acids; 4, methyl esters of Dutch beef tallow fatty acids; and 5, mixture of all stereoisomers of synthetic 11,15-octadecadienoic acid methyl ester.

TABLE II

Amounts Assessed by GLC of Fragments of Tallow Fatty Acid Methyl Esters, Obtained by Ozonolysis[a]

Carbon number and type of fragment	TLC bands									
	Australian					Dutch				
	II	III	IV	V	VI	II	III	IV	V	VI
Aldehydes										
C_3 A		M	S	S	M		S	S	M	S
C_6 A			M					L		
C_7 A	L	M				L	M			
C_9 A		L					L			
Dialdehydes										
C_3 DA			[b]		[b]			[b]		[b]
C_4 DA		S	S	S			S	S	S	
C_5 DA			S	S				S	S	
Aldehydic esters										
C_9 AE		L	L		L		L	L		M
C_{10} AE			S	S				S	S	
C_{11} AE	L	M	M	M		L	M	M	L	

[a] Abbreviations: L, large; M, medium; S, small. [b] Skew peak.

C_7 A). Band III contained much oleic acid (C_9 AE + C_9 A) and a reasonable amount of 9-*cis*-hexadecenoic acid (C_9 AE + C_7 A), as presumed. Band III contained in addition a small amount of 11-*trans*,15-*trans*-octadecadienoic acid (C_{11} AE + C_3 A + C_4 DA).

Band IV contained much normal linoleic acid (C_9 AE + C_6 A + C_3 DA), a reasonable amount of 11-*cis*(*trans*), 15-*trans*(*cis*)-octadecadienoic acids (C_{11} AE + C_3 A + C_4 DA), and a small amount of 10-*cis*(*trans*), 15-*trans*(*cis*)-octadecadienoic acids (C_{10} AE + C_3 A + C_5 DA).

Band V contained a mixture of 10-*cis*,15-*cis*- and 11-*cis*,15-*cis*-octadecadienoic acids (C_{10} AE + C_3 A + C_5 DA; C_{11} AE + C_3 A + C_4 DA respectively).

Band VI contained linolenic acid (C_9 AE + C_3 A + C_3 DA).

Isolation of Decomposition Aldehydes From a Model Mixture of Isomeric 11,15-Octadecadienoic Acids

As it was established that our Australian and Dutch tallows contained 11,15-octadecadienoic acids (all four possible isomers), it seemed interesting to study whether the aldehydes as isolated from tallow could really be isolated from those acids.

A small amount of the previously mentioned isomerized mixture of methyl 11,15-octadecadienoate, containing all possible isomers, was oxidized at 37 C in a Warburg apparatus to a peroxide value of 4 meq./kg (ca. 0.06% of hydroperoxides). The oxidized methyl esters were heated at 100 C for 1½ hr in a N_2 atmosphere to convert the hydroperoxides into aldehydes.

This mixture was degassed at 40 C/10^{-6} mm Hg residual pressure for 5 hr (8). The degassing products (i.e., the aldehydes) were transformed to their 2,4-dinitrophenylhydrazone (DNPH) by the method of Haverkamp Begemann and de Jong (9). The

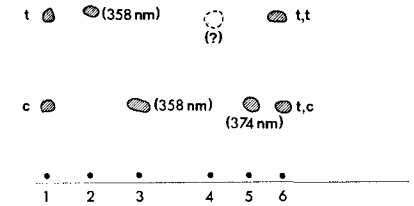

FIG. 2. TLC of degassing products of isomerized 11,15-octadecadienoic acid: 1, mixture of synthetic 4-heptenal-DNPHs; 2–5, isolated DNPHs of decomposition products of model isolinoleic acid; and 6, mixture of synthetic 2,6-nonadienal-DNPHs.

DNPHs obtained were analyzed by a new method of analyses according to Meijboom (10): they were separated with a Kieselguhr/Carbowax system. Those present between the R_f values of pentanal and hexanal DNPH were scraped off. After extraction the spot was rechromatographed on Silica gel G, together with four model substances (DNPHs of 4-*cis*- and 4-*trans*-heptenal and those of 2-*trans*,6-*cis*- and 2-*trans*,6-*trans*-nonadienal). The spots showing the same R_f value as the model substances were scraped off, extracted and rechromatographed on Silica gel G impregnated with $AgNO_3$, again together with the four model aldehyde DNPHs (Fig. 2). UV spectrophotometric measurement of the spots substantiated the findings by TLC.

Figure 2 shows that the DNPH spots obtained from the degassing products of the oxidized 11,15-octadecadienoic acids correspond with those of the model aldehyde DNPHs, in three cases, both in R_f value and λ_{max}. There was no substantial amount of 2-*trans*,6-*trans*-nonadienal DNPH to be measured by UV spectrophotometry.

Nevertheless it is evident that 11,15-octadecadienoic acid isomers (all four isomers) are the main precursors of the four aldehydes isolated from tallows. Because of the slight amount of 10,15-octadecadienoic acids analyzed in tallows, this acid can only serve as a secondary precursor for these aldehydes.

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