thetic acid was identical in rotation to the 9-hydroxystearic acid derived from the *Strophanthus* acid. The latter was thereby proved to have the D-configuration. We have similarly shown that dimorphecolic acid has the D-configuration (11) and, since the sign of rotation of the 9-hydroxystearate from the *Calendula* hydroxy acid is also negative it follows that it must have the D-configuration, i.e. (*S*) in the Cahn-Ingold-Prelog system (18).

Discussion

We have shown that the hydroxy acid which comprises some 5% of the total fatty acids of *Calendula officinalis* seed oil is D-(+)-9-hydroxy-10,12-octadecadienoic acid, one of the double bonds being *cis* and the other *trans*. This acid therefore is geometrically isomeric with dimorphecolic acid, the 9-hydroxy-*trans, trans*-10,12-octadecadienoic acid from *Dimorphotheca* oil. Like *Dimorphotheca* oil, *Calendula* oil does not contain any of the analogous 13-hydroxy-9,11-octadecadienoic acid isomer, as is the case with several other seed oils (2,8). Since this work was completed we have learned that the Peoria group have found what is probably the same acid amounting to some 65% of the acids of another species of the family *Compositae* (19).

We have not been able to determine if the conjugated diene system is *cis*-10,*trans*-12 or *trans*-10, *cis*-12 but we consider the latter configuration to be more likely from a consideration of possible biogenetic pathways. Thus, we (8,20) and others (3,4,15) have suggested in the past that these hydroxydienoic acids may be formed in nature from linoleic acid and that the structural correspondence of the hydroxydienoic isomers and the two series of conjugated trienoic acids (8,10,12- and 9,11,13-) suggests a common biosynthetic mechanism. These ideas have recently been elaborated by Gunstone (21) who proposes a generalised series of possible biological pathways to the various conjugated polyethenoid acids presently known to occur naturally. Although we consider that the 11-hydroxy-9,12-octadecadienoic acid intermediate proposed by Gunstone is neither necessary nor likely we concur with him in the belief that most of these conjugated unsaturated acids are derived from linoleic acid. If this is so, and we are currently engaged on biosynthetic experiments to try to clarify this (20), then one of the double bonds of linoleic acid must migrate into conjugation with the other. In so doing this bond will adopt the *trans*-configuration, as suggested by Gunstone (21) and as is the case in chemical autoxidation and in lipoxidase oxidation, and the bond which has not migrated will retain its *cis*-configuration. The 9-hydroxydiene acid would therefore have the *trans*-10,*cis*-12 configuration. That the 8,10,12-conjugated trienoic acid, which is the major component of *Calendula* oil and which may be considered to be derived from the hydroxydiene acid by dehydration, has the *trans*-10,*cis*-12 grouping in its conjugated system lends support to our conclusion that the *Calendula* hydroxy acid is D-(+)-9-hydroxy-*trans*-10,*cis*-12-octadecadienoic acid.

ACKNOWLEDGMENTS

Technical assistance, S. G. Welch and E. W. Hammond.

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[Received July 30, 1965—Accepted September 27, 1965]

Anatomical Variation in Fatty Acid Composition and Triglyceride Distribution in Animal Depot Fats[1]

G. K. CHACKO and E. G. PERKINS, The Burnsides Research Laboratory, University of Illinois, Urbana, Illinois

Abstract

The fatty acid composition and glyceride distribution of fatty acids in pork, beef and lamb depot fats from different localities within the same animal were determined by a combination of gas liquid chromatography and lipase hydrolysis techniques. The glyceride distribution was calculated according to the method of Vander Wal, based on the 1, 3-random and 2-random distribution pattern. Both fatty acid composition and glyceride structure were found to vary depending on the position within the animal from which the depot fat was obtained.

THE FATTY ACID COMPOSITION and glyceride type distribution of natural fats have been extensively studied by Hilditch and co-workers (1). These studies involved classical methods of fractional crystallization from suitable solvents followed by chemical analysis. The discovery that primary hydroxyl groups of glycerol esters of fatty acids are specifically cleaved by pancreatic lipase (2,3) permitted the detailed structural analysis of natural fats. Many theories of triglyceride distribution have been proposed and defended (4,5); and the 1, 3- random and 2- random distribution hypothesis of Vander Wal (5) has been shown to be applicable to a large number of depot fats. He proposed a method for calculating the distribution of saturated and unsaturated acyl groups in fats (5) from pancreatic lipase hydrolysis data. It is recognized from these studies, and those of others that the 2- position in the molecule of natural fats is generally occupied by a proportion of acyl groups

[1] Presented at the AOCS meeting in Chicago, October, 1964.

TABLE I

Fatty Acid Composition of Beef Fats

Anatomical position	Back fat layer, rump						Perinephric			Visceral pericardial			Fat attached to ribs		
	I 1 cm depth, bottom portion			II[a] 1 cm depth, top portion			III			IV			V		
Fatty acid	$C_{1,2,3}$	$C_{1,3}$	C_2[b]	$C_{1,2,3}$	$C_{1,3}$	C_2[b]	$C_{1,2,3}$	$C_{1,3}$	C_2[b]	$C_{1,2,3}$	$C_{1,3}$	C_2[b]	$C_{1,2,3}$	$C_{1,3}$	C_2[b]
14:0	6.9	2.5	15.7	5.2	3.4	8.8	5.2	3.2	9.2	2.1	2.0	2.3	4.4	2.6	8.0
14:1	1.5	0.7	3.1	2.5	1.2	3.9	0.6	0.5	0.8	0.7	1.1	0.0	1.2	0.6	2.4
16:0	33.1	39.4	20.5	33.7	39.9	21.3	35.3	38.4	29.1	26.9	35.9	8.9	32.5	33.4	30.7
16:1	5.4	2.6	11.0	6.0	5.0	8.0	2.5	3.2	1.1	5.0	1.8	11.4	4.0	4.7	2.7
18:0	14.0	21.5	0.0	9.5	13.6	1.3	20.3	31.2	0.0	34.2	43.3	16.0	15.7	23.4	0.3
18:1	39.1	33.3	50.7	42.3	35.8	55.3	36.2	22.9	62.8	29.0	15.9	55.2	39.9	33.5	52.7
18:2	.....	.....	.....	0.9	0.7	1.3	.....	.....	.....	2.2	.....	.....	1.5	0.9	2.7

[a] Sample from another beef.
[b] Calculated values.

TABLE II

Fatty Acid Composition of Pork Fats

Anatomical position	Back fat layer, rump						III Perinephric fat			IV Flank fat		
	I 1 cm depth, bottom portion			II 1 cm depth, top portion								
Fatty acid	$C_{1,2,3}$	$C_{1,3}$	C_2[a]	$C_{1,2,3}$	$C_{1,3}$	C_2[a]	$C_{1,2,3}$	$C_{1,3}$	C_2[a]	$C_{1,2,3}$	$C_{1,3}$	C_2[a]
14:0	1.4	1.0	2.2	2.1	0.6	5.1	1.7	0.6	3.9	2.0	0.7	4.6
16:0	31.6	15.9	63.0	29.2	10.2	67.2	30.6	9.7	72.4	24.3	11.1	50.7
16:1	2.9	1.9	4.9	3.3	2.0	5.9	2.9	2.2	4.3	5.2	2.3	11.0
18:0	13.4	21.0	−1.8	18.2	18.6	17.4	11.3	14.3	5.3	16.9	17.1	16.5
18:1	38.8	45.2	26.0	31.2	46.8	0.0	41.8	58.3	8.8	38.0	53.5	7.0
18:2	11.9	12.5	10.7	16.0	21.8	4.4	11.2	15.0	3.6	13.6	15.4	10.0

[a] Calculated values.

different from those of the 1, 3- positions. Thus, in beef tallow. the 1, 3- positions are esterified with greater percentages of palmitic and stearic acids than are found in the 2- position, whereas myristic and oleic acids are found in greater proportions in the 2- position. This preference or specificity of individual fatty acids has been defined as the percent positive deviation from random distribution and expressed mathematically by Vander Wal et al. (6).

Anatomical variation in fatty acid composition and glyceride type distribution has not been studied extensively. Hilditch and co-workers (1) have determined the total fatty acid composition of the trisaturated glycerides of pork fat samples obtained from different localities within the animal and have reported variation in the fatty acid composition. Using acetone fractionation methods and iodine value determination of the total fat and the fatty acids liberated by pancreatic lipase hydrolysis, Savary et al. (3) showed that the preferential attachment of saturated fatty acids at the 2- position of glyceride molecules in pork fat is not affected by anatomical location, even though the fatty acid composition does differ. Clement et al. (7) also showed that there are differences in composition between triglycerides of perirenal fat and those of perigenital tissue. Hanahan (8) suggested that "there is the distinct possibility, particularly of the animal glycerides that the distribution may vary not from one species to another but from one tissue to another." In the present study, therefore, fat samples obtained from different localities of the same animal of different species—beef, sheep, and swine—have been investigated and the fatty acid composition and glyceride type distribution compared.

Experimental

Fresh samples of fat tissue from various positions of the animals were obtained from the Animal Science Department of the University of Illinois. The samples were homogenized and extracted with Skelly F, dried over sodium sulfate and solvent removed using a rotary vacuum evaporator. Methyl esters of the whole fat were prepared by interesterification with anhydrous methanol containing 2% H_2SO_4. Methyl esters so obtained were analyzed by gas-liquid chromatography (GLC) as reported elsewhere (9). The composition of the individual methyl ester was calculated by the triangulation technique. Minor component fatty acids occurring at levels under 1% were not included in the analyses[2]. The procedure employed in this study for pancreatic lipase hydrolysis of groups in the 1- and 3- positions of triglycerides and the subsequent preparation of the methyl esters of the liberated acids using diazomethane has been published by Ast and Vander Wal (10)[3]. However, the fatty acids obtained by pancreatic lipase hydrolysis after methylation with diazomethane were directly analyzed by (GLC) without further separation. The proportions of glyceride types and isomeric forms were calculated from the total fatty acid composition of the whole fat and from those fatty acids present at the 1, 3- positions of the corresponding glyceride molecules obtained by pancreatic lipase hydrolysis (5).

Results and Discussion

Comparison of the results obtained between the inner- and outermost portion of the back fat layer indicates that the inner side of this fat layer contained more myristic acid than the outer portion and was more saturated. The composition of those acids present at the 1, 3- and 2- positions of triglycerides obtained from various bovine fat deposits is shown in Table I. Perinephric fat contained considerably more palmitic acid in the 2- position than the depot

[2] Minor component fatty acids when not included in the calculation will not contribute a substantial amount of error since they will occur in both S and U forms in similar amounts.
[3] Fatty acids released by pancreatic lipase from the 1,3- positions have been shown to be representative by Luddy et al. (14). The report by Desnuelle and Savary (15) also indicates that there was probably little selective hydrolysis between the fatty acids occurring as major components (16:0, 16:1, 18:0, 18:1, 18:2).

TABLE III

Fatty Acid Composition of Pork Fats

Anatomical position:	Back fat layer, shoulder						Back top [a]			Back Bottom [a]		
	V Upper 1 cm			VI Lower 1 cm			VIII			IX		
Fatty acid	$C_{1,2,3}$	$C_{1,3}$	C_2 [b]	$C_{1,2,3}$	$C_{1,3}$	C_2 [b]	$C_{1,2,3}$	$C_{1,3}$	C_2 [b]	$C_{1,2,3}$	$C_{1,3}$	C_2 [b]
14:0	1.7	0.4	4.3	1.9	1.3	3.1	2.0	0.9	4.2	2.2	0.2	6.2
16:0	24.4	6.0	61.2	25.9	15.7	46.3	27.1	10.0	61.3	29.2	9.8	68.0
16:1	2.8	1.6	5.2	2.8	2.1	4.2	4.0	2.8	6.4	3.5	1.8	6.9
18:0	11.9	12.0	11.7	15.0	22.4	0.2	11.0	15.1	2.8	12.7	17.1	3.9
18:1	46.5	66.7	6.1	39.8	46.8	25.8	44.4	54.2	24.8	41.5	56.5	11.5
18:2	10.5	11.9	7.7	14.5	11.7	20.1	11.4	16.1	2.0	11.0	14.7	3.6
18:3	1.2	1.0	1.6									

[a] III and IX are from another animal.
[b] Calculated values.

TABLE IV

Fatty Acid Composition of Sheep Fats

Anatomical position	Outer side of back fat layer (1 cm depth)			Inner side of back fat layer (1 cm depth)			Fat attached to ribs inside of cavity		
Fatty acid	$C_{1,2,3}$	$C_{1,3}$	C_2 [a]	$C_{1,2,3}$	$C_{1,3}$	C_2 [a]	$C_{1,2,3}$	$C_{1,3}$	C_2 [a]
14:0	2.3	2.2	2.5	2.4	1.9	3.4	2.1	2.1	2.1
15:0	0.3	T	0.9	0.3	0.3	0.3	0.4	0.2	0.8
16:0	21.0	24.9	13.2	20.8	27.1	8.3	25.4	34.3	7.6
16:1	1.0	0.9	1.2	1.0	0.6	1.8	1.2	0.9	1.8
17:0	0.8	0.7	1.0	1.2	1.5	0.6	1.6	1.3	2.2
18:0	29.5	33.3	21.9	35.5	43.4	19.7	32.1	36.2	23.9
18:1	43.5	35.2	60.1	37.7	25.0	63.1	36.8	24.6	61.2
18:2	1.4	1.7	0.8	T	T	T	T	T	T

[a] Calculated values.

fat (29% compared to 20%) and contained about 63% of oleic acid compared to the 50%–55% in the back fat layer. The composition of visceral pericardial fat is similar to the fat found in the back fat layer with the exception of myristic acid. The high content of myristic acid found in the sample of back fat may be expected because of a possible fluidizing effect of this acid in the 2- position as suggested by Vander Wal (12). The fatty acid composition of rib fat is intermediate between that found for perinephric fat and outer depot fat. These variations indicate that fatty acid composition is somewhat dependent upon the anatomical position of the fat in the animal. In the case of beef fats, myristic, palmitic, oleic and stearic acids showed considerable variation.

In pork fat the general pattern of distribution of fatty acids is maintained in all the samples (Tables II, III). The lower portion of the back fat layer contained 26.0% oleic acid and 10.7% linoleic acid in the 2- position (Table II) while the flank fat contained 50.7% palmitic acid and 10% linoleic acid. Perinephric fat contained 8.8% oleic and only 3.3% linoleic acid in the C-2 position. Thus, the total palmitic acid content varies from 24.3%–31.6%, stearic from 11%–88.2%, and oleic from 31.2%–46.5%. The general pattern of distribution among the hydroxyl groups is that myristic, palmitic and palmitoleic acids are preferentially attached to the 2- position. The fatty acid distribution in the case of sheep fats indicated a pattern similar to those of beef fats (Table IV).

Recent studies, especially by the pancreatic lipase hydrolysis technique have demonstrated, in general, that in animal fats, the 2- position of the glyceride molecules are occupied by unsaturated acyl groups in higher proportions than by saturated groups, and the 1, 3- positions in higher proportions by saturated acyl groups than by unsaturated groups. An exception to this general distribution was found in pork fats where the 2- positions are occupied by higher proportions of saturated fatty acids, especially palmitic acid. In their search to see whether this exception is unique only for pork fats or whether it is a general characteristic of even-toed animals, Mattson et al. (11) found that fats of European and American wild boar and those of two species of peccary also fall into this category.

In terms of total saturated and unsaturated fatty acids, perinephric and visceral pericardial bovine fats have higher proportions of saturated fatty acids, as compared to fats from other parts of the body. This difference is due predominantly to an increase in stearic and a decrease in oleic acid. In the various pork fat samples, perinephric fat showed a higher percentage of total saturated fatty acids.

The glyceride type distribution of several beef fat samples calculated from the pancreatic lipase hydrolysis data is given in Table V. In contrast to the considerable difference in the amount of total saturated and unsaturated fatty acids among samples obtained from different anatomical locations, there is little difference in the concentration of trisaturated glyceride among samples. However, in the case of perinephric and visceral pericardial fats, it can be seen that trisaturated glyceride concentrations are actually a little higher and the triunsaturated glycerides considerably less. It may also be significant that the symmetrical monounsaturated disaturated glyceride concentration is considerably higher in perinephric and visceral pericardial fats than in that of the fats obtained from other anatomical positions. The U_3 content of perinephric and visceral pericardial fats was considerably lower than that of the other samples.

Glyceride type distribution of pork fat (Table VI) samples also show definite variations in their concentration, according to the area from which the fat was obtained. Back fat contained a higher proportion of SSU than fats taken from other positions; perinephric fat contained considerably less U_3 than did the other samples.

An examination of the glyceride types of sheep fat (Table VII) indicates that fats obtained from the inner side of the animal's back fat layer have a higher trisaturated glyceride content and lower triunsaturated glyceride content when compared to that from the outer side of the animal. Only small difference can be seen between the diunsaturated and

TABLE V

Glyceride Type Distribution of Beef Fat

Glyceride type	SSS	SUS	SSU	USU	UUS	UUU
Locality of fat						
Bottom back rear	14.6	25.2	16.9	4.9	29.7	8.8
(random calculated)	17.1	13.7	27.4	11.0	22.0	8.8
Back bottom rear[a]	13.9	27.2	14.0	3.5	30.6	7.6
(random calculated)	13.1	12.7	25.4	12.3	24.6	11.9
Perinephric fat	20.2	32.8	15.1	2.8	26.3	4.5
(random calculated)	35.3	14.6	29.2	6.1	12.1	2.5
Visceral pericardial fat	15.4	41.1	10.4	1.7	27.2	4.5
(random calculated)	25.1	14.7	29.4	8.6	17.2	5.0
Rib cavity	13.7	21.6	18.8	6.4	29.4	10.1
(random calculated)	15.2	13.3	26.6	11.6	23.2	10.1

[a] From another animal.

TABLE VI

Glyceride Type Distribution in Pork Fat

Anatomical position	SSS	SUS	SSU	USU	UUS	UUU
Bottom rear back	8.0	3.4	31.4	30.8	13.3	13.0
(random calculated)	9.9	11.6	23.0	13.6	27.2	15.4
Kidney	7.7	8.9	37.3	44.7	4.3	5.1
(random calculated)	12.1	12.7	25.4	12.6	25.2	12.8
Top rear back sirloin	4.9	1.1	38.4	46.6	8.6	10.5
(random calculated)	8.6	10.9	21.8	13.8	27.6	17.4
Flank fat	5.9	2.3	29.5	36.3	11.6	14.3
(random calculated)	8.1	10.6	21.2	13.8	27.6	18.3
Back fat layer (shoulder) Upper 1 cm	2.6	0.7	23.0	51.4	6.8	15.2
(random calculated)	5.9	9.3	18.6	14.5	29.0	22.7
Back fat layer (shoulder) Lower 1 cm	7.7	7.7	22.6	18.2	23.9	18.3
(random calculated)	7.9	10.5	21.0	14.0	28.0	18.6
Back top[a]	4.5	2.1	26.2	37.5	12.2	17.9
(random calculated)	6.5	9.7	19.3	14.4	28.7	21.4
Back bottor[a]	5.8	1.6	30.8	41.2	8.8	11.7
(random calculated)	8.5	10.8	21.7	14.0	28.0	17.6

[a] Different animal.

triunsaturated triglyceride composition of sheep fat.

In contrast to the recent report of Barford et al. (13) the results obtained in the present study indicate that considerable differences exist between the percentages of any given fatty acid located in the 2- position of the glycerol moiety among the various tissues examined. A comparison of the results obtained when the glyceride distribution was calculated from pancreatic lipase hydrolysis data and that which would be expected from a random distribution indicates that the depot fats examined here are distributed in a nonrandom manner.

TABLE VII

Glyceride Type Distribution in Sheep Fat

Glyceride type	SSS	SUS	SSU	USU	UUS	UUU
Locality of fat:						
Outer side of back fat layer						
(1 cm depth)	14.4	24.0	17.6	5.3	29.4	8.9
(random, calculated)	15.7	13.6	26.8	11.5	23.0	9.8
Inner side of back fat layer						
(1 cm depth)	17.7	37.6	12.2	0.2	25.9	4.9
(random, calculated)	21.8	14.4	28.8	9.5	19.0	6.3
Fat attached to rib inside of rib cavity	20.4	34.6	14.0	4.1	23.8	4.1
(random, calculated)	23.7	14.6	29.2	9.0	18.0	5.5

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[Received April 7, 1965—Accepted August 2, 1965]

Dietary Fatty Acids: Their Metabolic Fate and Influence on Fatty Acid Biosynthesis[1]

NESTOR R. BOTTINO, ROBERT E. ANDERSON and RAYMOND REISER

Department of Biochemistry and Nutrition, Texas A&M University, College Station, Texas

Abstract

Adult rats fed a low-fat diet or diets containing 15% of either tripalmitin, triolein or trilinolein were injected intraperitoneally with H^3-labeled acetate. Those which received fat were also given by mouth, simultaneously with acetate, the 1-C^{14}-labeled sodium salt of the respective dietary fatty acid. The fate of the tagged material was followed by time-spaced biopsies of subcutaneous adipose tissue and by collection of the expired $C^{14}O_2$.

After 72 hr, 51, 64, and 52% of the dietary palmitate, oleate, and linoleate, respectively, were catabolized, as indicated by the corresponding percentages of the label having been excreted as $C^{14}O_2$. Dietary linoleate was relatively less incorporated into body triglycerides than palmitate and oleate. Animals ingesting diets of 15% triolein had only about one-half the amount of phospholipids in their tissues as had the other groups.

The distribution of both the C^{14} and H^3 labels in the tissue triglycerides showed that all diets containing fats decreased fatty acid synthesis but did not inhibit conversion of palmitate to oleate. Conversions of oleate or linoleate appeared to be through acetate. As a result of these factors, the fatty acid composition of the tissue triglycerides after 3 months' ingestion of tripalmitin was essentially the same as that of the low-fat group, whereas the ingestion of triolein produced triglycerides with a very high content of oleic acid. Trilinolein ingestion produced effects similar to triolein but to a less pronounced degree.

Both the respiratory $C^{14}O_2$ and the C^{14}- and H^3-labeled fatty acids in subcutaneous adipose tissue exhibited a second rise in specific activity 12 to 24 hours after the administration of the label.

[1]Presented at the AOCS meeting, Chicago, 1964.