ume proved to be the best solvents, and for palmitic and stearic acids the most satisfactory results were obtained with either benzene or a two-to-one mixture by volume of benzene and acetone. Since the molecular compounds must be crystallized from concentrated solutions, the separation of the crystals from the mother liquor must be made by centrifugation. The recrystallized fatty acid-acetamide compounds provide a convenient intermediate for the preparation of pure acid amides free from homologs.

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Some Recently Discovered Constituents of Animal Fats[1]

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UNTIL RECENTLY it was believed that fatty acids in natural fats were straight-chain molecules with an even number of carbon atoms. The occurrence of *iso*-valeric acid in the depot fats of dolphins and porpoises was regarded as a solitary exception (1). Whereas branched-chain fatty acids have been found in the past in the lipids of certain bacteria by Anderson (2) and more recently in wool grease by Weitkamp (3), these lipids were esters of high molecular alcohols and not glycerides.

Branched-chain Fatty Acids in Animal Fats. Accordingly isolation of branched-chain fatty acids from butterfat by Hansen and Shorland, first reported in 1950 (4), created widespread interest. This discovery originated from an investigation of the efficiency of separating liquid and solid methyl esters of butterfat by crystallization from acetone and fractional distillation. When concentrates of C_{18} liquid esters were oxidized with potassium permanganate in acetone, there was a residual neutral product which was saturated and yet liquid at room temperature. The product was resolved into several fractions, one of which suggested the presence of branched-chain fatty acids. In the course of further investigations Hansen and Shorland obtained evidence of the occurrence of several branched-chain fatty acids in butterfat (5a,b, 6a,b, 7a,b) and this was followed by the isolation of a number of such acids from the depot fats of oxen and sheep (8a,b,c,d,e).

The procedure for the isolation of these acids consisted in earlier studies in combining fractional distillation with hydrogenation and low temperature crystallization from acetone, which separates branched-chain from straight-chain saturated acids. However, in view of Hofmann and Lucas' findings (9) that hydrogenation could lead to the formation of branched-chain acids if the original product contained a cyclopropane ring, careful fractional distillation has been employed rather than hydrogenation. With the use of highly efficient fractionating columns the small amounts of branched-chain fatty acids present in natural fats could be sufficiently resolved to permit their subsequent purification by low temperature crystallization alone. The types and approximate amounts of branched-chain acids found in butterfat and depot fats of oxen and sheep are shown in Table I(a).

The Occurrence of Straight-chain Odd-carbon Numbered Acids in Ox, Sheep, and Butterfat. An essentially similar technique led to the isolation of straight-chain odd carbon numbered fatty acids in the above mentioned fats by the workers of this laboratory (7a,b, 10a,b, 11). The existence of such acids, and especially of margaric acid, in natural fats had been repeatedly reported in the past but never substantiated, as might be seen from Ralston's (12) following statement:

> Heptadecanoic acid does not occur in the natural fats and oils, although its presence has often been reported, and it has been only within recent years that such names as margaric acid and daturic acid have ceased to be the subject of scientific controversies. All the naturally occurring heptadecanoic acids which have been described, and subsequently investigated, have been shown to consist of mixtures of palmitic and stearic acids. Because of the frequent mutual occurrence of palmitic and stearic acids in fats, it is not surprising that a mixture of them has frequently been identified as a pure compound.

The identification of the above-mentioned acids was based on greatly improved methods of fatty-acid analysis by fractional distillation and low temperature crystallization, on measurements of long crystal spacings by X-ray diffraction, determination of mixed melting points with pure synthetic acids, and the like. Incidentally a qualitative test for the presence of saturated *n*-odd numbered fatty acids might be based on their characteristic property of shrinking from glass on cooling, which is not shown to any comparable extent by even-numbered fatty acids and their mixtures. Table I(b) shows the various straight-chain odd-numbered acids isolated up till now. It might be noted that their occurrence is not restricted to the saturated acids only.

The vapor-liquid chromatographic method of James and Martin (13), based on the automatic titration of fatty acids, was applied to the detection and estimation of both branched-chain and odd-numbered straight-chain volatile acids in the ox fat on a micro scale, and a consecutive series of these acids from C_2–C_{10} has been reported by Hansen and McInnes (14). Furthermore results obtained by Shorland and co-workers have been confirmed by James and Martin (15) with the use of their new chromatographic apparatus based on vapor density measurements, which

[1] Presented at the Fall Meeting of the American Oil Chemists' Society, Philadelphia, Pa., Oct. 10–12, 1955.

TABLE I

Branched-chain and Odd-Numbered Straight-chain Fatty Acids in Ox, Sheep, and Butterfat

	Number of carbons	Estimated percentage	References
a) Branched-chain acids			
Iso-acids $\begin{matrix}CH_3\\ \diagdown\\ \quad CH\cdot(CH_2)_nCOOH\\ \diagup\\ CH_3\end{matrix}$	13, 14, 15, 16, 17	0.5–1	(5a, 6b, 7a, 8c,d,e)
Anteiso-acids $CH_3\cdot CH_2\cdot \underset{\displaystyle CH_3}{\underset{\vert}{CH}}\cdot(CH_2)_n\cdot COOH$	13, 15, 17	0.5–1	(5a, 7a,b, 8a,b,c)
Di- and polybranched-chain acids	17, 18, 20	Traces	(5b)
b) Odd-numbered straight-chain acids			
Saturated	11, 13, 15, 17, 19	1.5–2	(7a,b, 10a,b)
Unsaturated	17 (possibly also 13, 15)	Traces	(11)

permits the estimation of higher fatty acids.

The Origin of Branched-chain and Odd-numbered Straight-chain Acids in the Fats of Ruminants. El Shazly (16) found that a series of straight- and branched-chain fatty acids C_2 to C_5 is produced by rumen bacteria from amino acids. Volatile branched-chain acids could be formed from valine, leucine, and *iso*-leucine present in the pasture by Strickland reaction (17) involving deamination and decarboxylation. These volatile fatty acids could then, by successive condensation of acetate units, give rise to the three types of branched-chain fatty acids, actually isolated by Shorland and co-workers, according to the following scheme:

1. Valine would lead to *iso*-butyric acid, which on condensation with acetate would give rise to *iso*-acids (*i.e.*, acids with two terminal methyl groups) containing an even number of carbon atoms.

2. Leucine *via iso*-valeric acid condensed with acetate would give rise to *iso*-acids with an odd number of carbon atoms.

3. *Iso*-leucine *via* 2-methylbutyric acid condensed with acetate would lead to *ante-iso*-acids (*i.e.*, acids with a methyl group attached to the second carbon but last) containing an odd number of carbon atoms.

Similarly propionic acid produced by rumen bacteria could lead to the formation of straight-chain odd-numbered fatty acids.

The Trans-unsaturated Acid Contents of Fats of Ruminants and Non-ruminants. Another result of bacterial action might be the occurrence of *trans*-unsaturated acids in certain animal fats. Small amounts of vaccenic (*trans*-octadec-11-enoic) acid were discovered by Bertram (18) in butter and depot fats of oxen and sheep as far back as 1928, but it was only recently that Swern, Knight, and Eddy (19), by using infrared spectroscopy, found 5–10% of *trans* mono-ethenoid acids in beef fat, and Brown and co-workers similar amounts in butterfat (20). Shorland and his group extended this investigation to depot fats of various animals (21) and showed that, whereas ruminants contained considerable amounts of *trans*-acids, non-ruminants and birds contained little or none (*cf.* Table II). This difference was explained on the basis of Reiser's observation (22) that an extensive hydrogenation was taking place in the rumen. Since hydrogenation is as a rule associated with *trans*-isomerization, the hypothesis was put forward that the presence of *trans*-unsaturated acids in the depot fat of ruminants is largely caused by the activity of rumen bacteria. The comparison between lipids of pasture and of some typical pasture-fed ruminants and non-ruminants (23), with particular reference to the contents of sheep's rumen (24), illustrates the point (Table III). The hypothesis of *trans*-acid formation by the mechanism outlined above obtained additional support by the observation that the depot fat of certain marsupials, such as quokka, which is known to possess a semi-ruminant digestion (25), contains up to 21% of *trans*-acids (*cf.* Table II). The very high content of *trans*-acids in the quokka might be attributed to the fast turnover in the digestive system of this animal as compared with ruminants proper. Accordingly hydrogenation in the quokka is less extensive and produces more *trans*-acids than in the ruminants, where it proceeds to a large degree to the saturated acid stage.

TABLE II

Trans-unsaturated Acids in Fats of Various Ruminants, Non-ruminants, and Marsupials

Species	Sample	Trans-acids as % of elaidic acid
a) Ruminants		
Ox	Perinephric	4.5
Sheep	Perinephric	11.2
Goat	Perinephric	6.8–11.1
Fallow deer	Perinephric	3.5
b) Non-ruminants		
Rat	Body fat	nil
Pig	Perinephric	0.9
Horse	Perinephric	nil
Rabbit	Abdominal cavity fat	nil
Pheasant	Abdominal and external tissue fat	nil
c) Marsupials		
Wallaby (*Thylogale eugene*)		18.1–19.2
Quokka (*Setonix brachyurus*)		21.0

Conclusions

The occurrence of branched-chain, straight-chain odd-numbered and *trans*-unsaturated acids is not restricted to ruminants, but their quantities in other animal and vegetable fats seem to be of a much smaller order. This lends support to the belief that the bacterial processes in the rumen are responsible, at least to some extent, for the formation of the three above-mentioned types of fatty acids in the milk and depot fats of ruminants, also for some unique properties which these fats might possess.

Summary

A series of branched-chain and odd-numbered straight-chain fatty acids has been recently isolated from ox, sheep, and butterfat. Substantial amounts of *trans*-unsaturated acids have been found in the depot fats of ruminants, but none or little in non-

TABLE III
Comparison Between Fatty-acid Composition of Pasture Lipids and of Depot Fats of Pasture-fed Animals

Source of fat	% Fatty acids										% Trans-acids
	Saturated				Unsaturated						
							C_{18}				
	C_{12}–C_{14}	C_{16}	C_{18}	C_{20}	C_{12}–C_{14}	C_{16}	Monoene	Diene	Triene	C_{20}	
Pasture (ryegrass)	1.8	10.6	1.5	0.4	0.7	4.1	4.6	11.6	62.8	1.9	trace
Wild rabbit	1.6	22.1	6.4	0.8	0.4	4.4	12.7	7.9	42.4	1.3	nil
Horse	2.4	29.7	4.3	0.2	1.4	6.5	32.5	3.8	16.1	3.1	nil
Ox	2.7	27.8	21.6	....	0.3	2.5	42.5	0.5	0.3	1.8	4.5
Sheep	3.8	25.0	22.2	0.7	0.5	1.7	44.2	trace	trace	0.9	11.2
Rumen contents of sheep	1.2	16.9	48.5	5.8	0.2	1.8	19.2	3.5	2.9	....	10.9

ruminants. Mechanisms of the formation of all these constituents based on the activity of rumen bacteria are suggested.

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Identification of Some Marine Oil Constituents by Chromatography[1]

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OF ALL THE LIPIDE MIXTURES of natural origin, marine oils are the most complex and the most difficult to analyze. Among the constituent lipides of marine oils are the fatty alcohols, ether-alcohols, hydrocarbons, waxes, triglycerides, various phospholipides, and vitamins A, D, and E. Present methods for the determination of these lipides require large amounts of material and are laborious, time-consuming, and uncertain. For the most part these procedures are modifications of methods used for the analyses of other fats and oils and are not very efficient even for their original purposes.

A method has been devised by Dieckert and Reiser (1) for the separation and identification of μg. quantities of various lipides on glass-fiber filter paper impregnated with silicic acid. The present study was designed to determine whether that method could be used to advantage in separating and identifying the lipide constituents of marine oils. The paper method is incomparably simpler than other methods where analysis rather than isolation is the objective.

Experimental

Reference compounds. The following compounds, dissolved in A.C.S. chloroform, 1 mg. per ml., were used as reference: winterized cottonseed oil[2] (for triglycerides), vitamin A alcohol, vitamin A palmitate, 7-dehydrocholesterol, cholesterol, vitamin D_3, hexadecanol, cholesteryl palmitate, hexadecyl acetate, hexadecyl palmitate, d-α-tocopherol, d-α-tocopheryl acetate, and squalene.

Solvent systems. The solvent systems used were: cyclohexane; 1% methanol in cyclohexane, v/v; 2% ethyl ether in iso-octane, v/v; 2 g. iodine in 2% ethyl ether in iso-octane, v/v; 1%, 4%, 10%, and 50% ethyl ether in petroleum ether,[3] v/v; 25% and 50% methanol in ethyl ether, v/v. The cyclohexane was technical grade; the methanol and ethyl ether were A.C.S. grade; the iso-octane was rectified by passage through a silica gel column; and the petroleum ether[3] was tested for peroxides before use.

Column. Silicic acid, $SiO_2 \cdot nH_2O$, powder, Baker reagent grade, was used in a column for the preliminary separations.

Paper. Glass-fiber filter paper[4] was impregnated with silicic acid for the paper chromatography by the method of Dieckert and Reiser (1, 3).

Spray reagent. Aqueous sulfuric acid reagent, 50%, v/v, was used as a spray with subsequent heating to char the lipide spots and make them visible on the paper.

Special reagents. Choline containing lipides were identified by the Dragendorff reagent spot test (5).

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[2] Wesson Oil, Wesson Oil and Snowdrift Sales Company, New Orleans, La.
[3] Commercial n-hexane, Skellysolve B, b.p. 60°–70°C., Skelly Oil Company, Kansas City, Mo.
[4] No. X-934-AH, heavy weight, H. Reeve Angel and Company, 52 Duane street, New York, N. Y.