The Volatile Acids of Mutton Fat

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Recent investigations on ox perinephric fat revealed the presence of a consecutive series of 'odd and even' *normal* volatile fatty acids (VFA) ranging from C_2 to C_{10} (Hansen & McInnes, 1954). This present paper reports the identification of the same series of VFA together with formic, *iso*butyric, *iso*-valeric and α-methylbutyric acids in the external carcass fat of sheep. Formic acid has not hitherto been reported as a constituent of natural fats, although it has been found in the venous and arterial blood of animals (Annison, 1954*b*). *Iso* acids with molecular weight lower than that of the C_{15} *iso* acid 13-methyltetradecanoic acid (Hansen, Shorland & Cooke, 1953) have not formerly been isolated from animal depot fats, except *iso*valeric acid, which was identified in the body and head oils of dolphins and porpoises (Lovern, 1934). Until the present no *anteiso* acid with a molecular weight lower than that of 10-methyldodecanoic acid (Hansen, Shorland & Cooke, 1954*a*) had been shown to occur in animal depot fats. In cow milk, however, all the even-numbered *normal* volatile acids (C_4–C_{12}) are present (Hilditch, 1947), as are a number of odd-numbered *normal*, *iso*, and *anteiso* acids of higher molecular weight (Shorland, Gerson & Hansen, 1955*a*, *b*; Hansen, Shorland & Cooke, 1954*b*; Hansen & Shorland, 1951.)

EXPERIMENTAL

In preliminary investigations on the VFA of mutton fat, the glycerides (24·0 kg.) from a sample of fat (G 99) were cold-saponified, acidified and steam-distilled, and the VFA were extracted from the distillate with ether. The ether extract was fractionated in a 60 cm. fractionating column packed with glass helices, and the fractions obtained were titrated with approximately 0·5 N-KOH with phenolphthalein as indicator, and the soap solutions taken to dryness. In order to regenerate the fatty acids the soaps were moistened with a little water, treated with an excess of $KHSO_4$ and 2 drops of 10 N-H_2SO_4 and extracted with chloroform containing 5% (v/v) *n*-butanol. An attempt was then made to use buffered partition chromatography as described by Moyle, Baldwin & Scarisbrick (1948), to separate and identify the saturated acids from C_2 to C_8. Quantitative results have not been provided for the acids isolated by this method, as they could not be completely eluted from the columns. In order to confirm the identification of these acids gas–liquid chromatography was employed, the soaps (denoted C) obtained by evaporation of the chloroform–butanol solutions from the buffered silica-gel columns being dissolved in water and made up to approximately 2 N solutions.

To obtain more exact information this work was continued with two further samples from the external carcass fat of sheep (K 7 comprising wethers, ewes and rams: K 40 wethers). In K 7 the fatty tissues were minced and heated with water on a water bath. After repeated washing with water, and drying to constant weight under vacuum at 100°, the extracted glycerides (wt. 9·784 kg., saponification equiv. 285·2, iodine value 86·4, acid value 0·56) were converted into soaps, acidified and steam-distilled, the distillate passing into receivers containing aqueous KOH. The distillate (20 l.) was taken to dryness on a water bath, and the soaps were redissolved in water (200 ml.). In order to remove the unsaponifiable material the solution was extracted with ether, after which the soaps were reacidified and steam-distilled. The distillate (7 l.) was collected as seven equal fractions, the first of which was titrated with 0·742 N-NaOH, evaporated to dryness on a water bath and then dissolved in water and made up to an approximately 4 N solution (denoted A). Owing to the presence of higher fatty acids the soaps obtained from the other six fractions could not be dissolved in water to give a 2 N solution. The soaps were redissolved in water (2 l.) containing $MgSO_4$ (50 g.), steam-distilled in the manner suggested by Friedemann (1938) to yield a distillate (1·5 l.) which was titrated as before, and the solution evaporated to dryness. The soaps were dissolved in water and made up to an approximately 2 N solution (denoted B). K 7 (A) and K 7 (B) required 20 and 32·2 ml. of 0·742 N-NaOH respectively. Samples (30 μl.) of each of the solutions (A), (B) and (C) were treated as prescribed by James & Martin (1952) for the micro-estimation of volatile acids isolated as their sodium or potassium soaps.

The sample K 40 (wt. 1·06 kg., saponification equiv. 289·1, iodine value 26·0, acid value 0·8) was treated in much the same manner, except that the soaps obtained by titrating the steam distillate (23 ml. of 0·466 N-NaOH) were dissolved in water and made up to 20 ml. in a standard flask. A portion (2 ml.) of this solution was then treated in a manner devised by one of us (McInnes, 1956). The free VFA obtained on acidifying the soaps with $KHSO_4$ were dissolved in ether and the anhydrous extract was made up to 10 ml. A portion (0·5 ml.) of the ethereal solution was then introduced into a modified gas–liquid chromatographic column. The ether used throughout the work was freshly distilled from a mixture of $FeSO_4$ and KOH.

Analysis of volatile fatty acids. The gas–liquid partition chromatography method of James & Martin (1952) was employed at a temperature of 137°, with Cellosolve (2-ethoxyethanol) in the heating jacket. The column length was 4 ft. and the liquid phase was silicone oil M.S. 550 (Hopkin and Williams Ltd.) containing 10% (w/w) of stearic

acid. The gaseous phase was N_2. The C_1–C_5 acids were checked by operating the column at a temperature of 100°; the column was loaded with three times the quantity of acids mentioned above in order that those present in small proportions could be estimated more accurately.

Synthetic mixtures of VFA (Eastman Kodak Co.) were used to check the retention volumes of the acids which were present in the external carcass fat of sheep. Unless stated otherwise, all percentages of VFA are expressed on a molecular basis.

RESULTS

By means of gas–liquid chromatography all the straight-chain VFA (both odd and even) from C_1 to C_{10}, together with *iso*butyric, *iso*valeric, α-methylbutyric and four unidentified acids, have been shown to be present in the external carcass fat of sheep. It can be seen from Table 1 that each acid is expressed as a percentage of the total concentration of VFA from C_1 to C_{10}. In K 7, it is likely that the value for formic acid is too low since it has been the experience in this *Laboratory* that the method suggested by James & Martin (1952) for the application of fatty acids on to a 4 ft. chromatographic column results in a loss of formic acid from the column when a temperature of 150° is used, and is not safe for quantitative work even at a temperature of 100°. This has been overcome by the new procedure mentioned above (McInnes, 1956). Combined results for K 7 (A) and K 7 (B) are also given in Table 1.

In the work now reported VFA were first detected in the steam distillate of mutton fat by buffered-partition chromatography (Moyle *et al.* 1948). The individual acids isolated by this method were checked by the gas–liquid chromatographic procedure of James & Martin (1952), and it was found that the acids had not been eluted in the order described by Moyle *et al.* (1948). This was attributed to the gradual deterioration of the non-adsorptive properties of the silica gel, as it had been kept in an air-tight bottle for approximately 9 months. It appeared, however, that the method of Moyle *et al.* (1948) could be used satisfactorily if a check were kept on the properties of the silica gel by chromatographing standard solutions of acids alongside the unknown samples. A further advantage of the gas–liquid chromatogram of James & Martin (1952) was that the method provided for the identification of all the *normal* saturated VFA from C_1 to C_{10}, whereas the buffered columns were limited to a range of C_2–C_8. In addition, the above method enabled all the isomers up to *n*-valeric to be estimated.

The total weight of VFA as determined from the graphs obtained by the gas–liquid chromatographic apparatus are approximately 1·25 g. (K 7) and 0·60 g. (K 40). These are approximately 0·01 and 0·06 % by weight respectively of the total fatty acids.

Four unidentified acids with retention volumes relative to *n*-butyric acid of 2·5, 5·74, 9·68 and 17·00 at 137° were found. These values suggest that they may be isomers, or mixtures of isomers, of *n*-hexanoic, *n*-heptanoic, *n*-octanoic and *n*-nonanoic acids respectively. As the fatty acids were not hydrogenated before they were steam-distilled, the possibility that these four acids could be unsaturated cannot be overlooked.

The steam-distillation procedure which is adopted for preparing a volatile-acid fraction free from higher fatty acids is unsatisfactory; it is not possible to ensure that any given volatile acid has been completely separated from the higher fatty acids, and no procedure is known to the authors that will give exclusively any given fraction of volatile acids. Comparing the volatile acids in K 7 (A) and K 7 (B) in Table 1, it will be seen that fraction B contains all the formic acid together with a fair proportion of the acetic acid. This suggests that when fatty acids are steam-distilled, most of the acids from C_3 to C_9 pass over in a comparatively small volume of

Table 1. *Molecular percentages of* C_1–C_{10} *fatty acids from the external carcass fat of sheep*

	K 7 (A)	K 7 (B)	K 7 (A) + K 7 (B)	K 40	G 99 (C)
Formic	—	40·3	27·4	37·0	Present
Acetic	16·2	56·2	43·3	39·0	Present
Propionic	0·8	—	0·3	1·4	Present
*iso*Butyric	1·0	—	0·3	0·4	Present
n-Butyric	5·3	3·1	3·8	1·8	Present
*iso*Valeric + α-Methylbutyric	1·2	—	0·4	0·4	Present
n-Valeric	1·6	—	0·5	1·0	Present
Unknown	Trace	—	Trace	Trace	—
n-Hexanoic	29·0	—	9·3	9·9	Present
Unknown	2·8	—	0·9	0·4	Present
n-Heptanoic	4·4	—	1·4	1·7	Present
Unknown	2·4	—	0·8	0·7	—
n-Octanoic	11·6	—	3·7	2·1	Present
Unknown	4·8	—	1·5	0·4	—
n-Nonanoic	4·4	—	1·4	1·0	—
n-Decanoic	14·5	0·4	5·0	2·8	—

distillate, formic and acetic acids being steam-distilled with more difficulty.

Since the glycerides of K7 and K40 were dried under vacuum at 100° the lower VFA would have been removed had they been present as free fatty acids. Furthermore, although glycerides containing formic acid can be hydrolysed by boiling with water, it would appear that under the conditions used in this investigation hydrolysis does not occur to any appreciable extent, and the VFA identified in K7, K40 and G99 are present as glycerides.

DISCUSSION

The occurrence of *n*-, *iso*- and *anteiso*-acids in the VFA from the external carcass fat of sheep, as shown by this work, is not unexpected, since it is known that acetic, propionic, *iso*butyric, *n*-butyric, *iso*valeric, α-methylbutyric and trace amounts of hexanoic acids are present in the rumen of sheep (Annison, 1954*a*). It is of interest, however, to find that there exist differences in the relative amounts of VFA in the carcass fat and the rumen. For example, *n*-hexanoic acid occurs to the extent of approximately 10% of the total VFA from the fat, although it is present only in trace amounts in the rumen. It can be seen from Table 1 that the even-carbon VFA (approximately 55% of total VFA) occur in the following descending order of molecular concentration: *n*-acetic, *n*-hexanoic, *n*-decanoic, *n*-butyric, *n*-octanoic, *iso*butyric. The odd-carbon acids, on the other hand, excluding formic acid, occur to the extent of less than 5% of the total VFA in mutton fat, the higher members *n*-heptanoic and *n*-nonanoic being present in the greater proportions. The work of Gray, Pilgrim, Rodda & Weller (1951) has shown that *n*-valeric acid can be synthesized from propionate in the rumen of sheep, and it is in accordance with modern views that the higher acids in this series are synthesized by the successive addition of acetate units to propionate.

The concentrations of the odd-carbon (0·35% approximately of VFA) and even-carbon (0·35% approximately of VFA) branched-chain acids *iso*-valeric, α-methylbutyric and *iso*butyric acids together account for only a very small proportion of the total VFA. Their presence in the depot fat, however, supports the suggestions of various workers (Velick & English, 1945; Weitzel & Lennert, 1951; El-Shazly, 1952; Shorland, 1953) that these acids are the precursors of the higher branched-chain acids found by this laboratory in animal depot fats in amounts greater than 1% by weight of the total fatty acids.

No explanation can be offered at present for the large proportion (approximately 37%) of formic acid in the VFA of mutton fat, although it is known to be present in the rumen of sheep (Gray, Pilgrim, Rodda & Weller, 1951, 1952) and the venous and arterial blood of animals (Annison, 1954*b*).

SUMMARY

1. By means of gas–liquid chromatography all the *normal* fatty acids, both odd and even, from C_1 to C_{10} have been shown to occur in the external carcass fat of sheep.

2. Two *iso* acids, *iso*butyric and *iso*valeric, and an *anteiso* acid, α-methylbutyric, as well as formic acid, have for the first time been shown to be components of an animal depot fat.

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