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Coincidence of *cis*- and *trans*-monoethylenic fatty acids simplifies the open-tubular gas-liquid chromatography of butyl esters of butter fatty acids

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Fatty acids of whole butterfat were converted to butyl esters and fractionated by thin-layer chromatography on silica gel impregnated with $AgNO_3$. Fractions of C_{10}–C_{18} fatty acids were examined on a bonded polyglycol open-tubular gas-liquid chromatography (GLC) column. Comparisons of fractions showed that butyl esters of *cis* and *trans* monoethylenic fatty acids coincided exactly for most bond positions. Polyglycol column GLC of butyl esters thus simplifies analysis of butterfat. Some monoethylenic acids with bond positions closer to the carboxyl group than major components such as *cis*-18:1*n*-9 did not resolve from these components. A *trans*-18:1*n*-3 fatty acid previously reported was not found, but *trans*-18:1*n*-6, *n*-5, *n*-4 and *n*-2 were confirmed from GLC properties. The fatty acids 12:1 and 14:1 were shown by TLC and GLC to be exclusively a single *cis* isomer with the ethylenic bond in the Δ9,10 position. The GLC evidence for the unusual vinyl bond in the 10:1*n*-1 of butterfats is reviewed. It is proposed that these three fatty acids are products of a desaturase through the animal biochemistry proper as distinct from the rumen biochemistry processes which lead to the multiplicity of isomers of 16:1 and 18:1 fatty acids.

INTRODUCTION

The basic problem in the gas-liquid chromatography (GLC) of esters of the fatty acids of butterfat has been the quantitative recovery of the esters of the shorter chain (C_4-C_{10}) fatty acids after esterification. Methyl esters can be used (Bannon *et al.*, 1985; Ulberth & Henninger, 1992) although the butyl esters offer a markedly improved recovery system (Iverson & Sheppard, 1977; 1986; Jensen, 1992). Recently it was noted that isopropyl esters of two positional isomers of monoethylenic C_{18} fatty acids gave enhanced resolution compared to methyl esters (Wolff & Vandamme, 1992), presumably due to the imbalance of the bond position relative to the carboxyl group altering volatility (Ackman, 1970). Since we had been examining butyl esters for recovery of the shorter chain fatty acids of butterfat (Chawla, Tau & Ackman, unpublished) it seemed desirable to see if the retention times of the butyl esters of the *trans* acids were also affected, relative to the corresponding esters of *cis* fatty acids, by the butyl alcohol moiety. The liquid phase already in use was the 'bonded' polyglycol Omegawax (Supelco Inc., Bellefonte, PA) which has advantages of simple chemistry and a stable polarity promoting interlaboratory comparisons of retention data (Ackman, 1986).

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MATERIALS AND METHODS

The butyl esters of the fatty acids of anhydrous butterfat were prepared by transesterification with BF_3 (12%) in *n*-butanol, with an equal volume of *n*-hexane, by heating at 100°C in a screw-capped centrifuge tube for 60 min. The recovered esters, dissolved in $CHCl_3$, were streaked on a silica gel TLC plate (Adsorbosil-5, Alltech Associates Inc., Applied Science Laboratories, Dearfield, IL) impregnated with $AgNO_3$ as described elsewhere (Ackman *et al.*, 1981*a*). The plate was developed in benzene:hexane (2:1) and ester bands were detected by spraying with 1% dichlorofluorescein in ethanol and marked while examining under UV light. There were three very distinct mobile bands (respective R_f values 0·78; 0·67 and 0·61 for No.4, No. 3 and No. 2, respectively) and a weaker band of R_f 0·44 (No. 1). The silica gel for each band was scraped off and ex-

tracted three times with $CHCl_3$:MeOH 1:1. The filtered solvent was removed on a rotary evaporator and the esters taken up in hexane for analysis by GLC. The Omegawax column, 30 m x 0·25 mm, was operated in a Model 8420 GLC unit (Perkin Elmer Corp., Norwalk, CT) with helium as the carrier gas at 12 psig. The injection and detector areas were held at 250°C and the initial injection was at 60°C. After 3 min, the oven temperature was raised at 10°C/min to 200°C and held there.

RESULTS AND DISCUSSION

Figure 1 shows part (C_{10}–C_{18}) of the gas-liquid chromatogram of the butyl esters of the fatty acids of whole butterfat. The peak for butyl oleate (18:1*n*-9; included in peak A) emerged at 27 min under the conditions employed. Among the lesser components some features to note are the single 14:1 and 16:1 peaks, the unsaturated group of C_{18} peaks, labelled A–F, and two components provisionally marked 'unknown', in the iso 11:0 position and just ahead of iso 13:0, respectively.

The most mobile TLC band (No. 4) gave an extremely clean baseline (Fig. 2) and included the esters of the straight-chain and methyl-branched longer-chain (C_{10}–C_{18}) saturated fatty acids. The anteiso-15:0 and anteiso-17:0 fatty acids exceeded the iso-15:0 and iso-17:0 acids as noted previously for ruminant milk fats (Ackman & Hooper, 1973). Slight skewing of the iso-17:0 peak suggested inclusion of a minor component. After examining band 3 (see below) it can be concluded that traces of *trans*-16:1, and of peaks A and F, are present in band 4. The small component (?) ahead of the major 16:1 peak in Fig. 1 also appeared to be present in band 4.

The second and less mobile band (No. 3) contained relatively more of the esters of the shorter chain fatty acids (C_4–C_{12}), but 14:0, 16:0 and 18:0 were still represented (Fig. 3). There was a major group of four peaks (Fig. 3, A–D), and one well-separated peak (F) following this group. The position of this band on the TLC plate strongly suggested *trans*-18:1 components. The leading edge of peak A coincides with the front on the major 18:1 peak in the butyl esters of whole butterfat and also with the front of the major peak of the *cis*-18:1 ester group (band 2; see below). From the published tabulations of butterfat and beef fat isomeric monoethylenic fatty acids (Table 1), it can be deduced that peak A is a composite of *trans*-18:1*n*-9 and earlier eluting *trans* isomers, peak B is *trans*-18:1*n*-7 (vaccenic acid), peak C is *trans*-18:1*n*-5 and peak D is *trans*-18:1*n*-4. Hay and Morrison (1970) suggest several minor *trans* isomers including 18:1*n*-6, *n*-5 and *n*-4, in butter fat. Discussion of the above group should therefore include the shoulder between B and C which is evidently *trans*-18:1*n*-6, but it is relatively minor in proportion. The identity of peak F, probably 10% of the total *trans* 18:1 isomer group, can be deduced from its GLC behaviour. On polar liquid phases, *trans*-18:1*n*-2 fatty acid would have an exaggerated increase in retention time compared to other positional isomers and especially the two adjacent (*n*-1, *n*-3) positional isomers (Gunstone *et al.*, 1967; Ackman, 1972). A *trans*-16-octadecenoic (18:1*n*-2) acid has been reported in both butter and beef fats (Table 1) and this makes the proposal that F is *trans*-18:1*n*-2 likely. However it is obvious that there is no peak for the *trans*-18:1*n*-3 of Table 1 in the position anticipated, which would be just after peak D (18:1*n*-4), according to Gunstone *et al.* (1976). To ontain a *trans* acid in the rumen system the bond must shift position, and 18:1*n*-3 is a natural bond

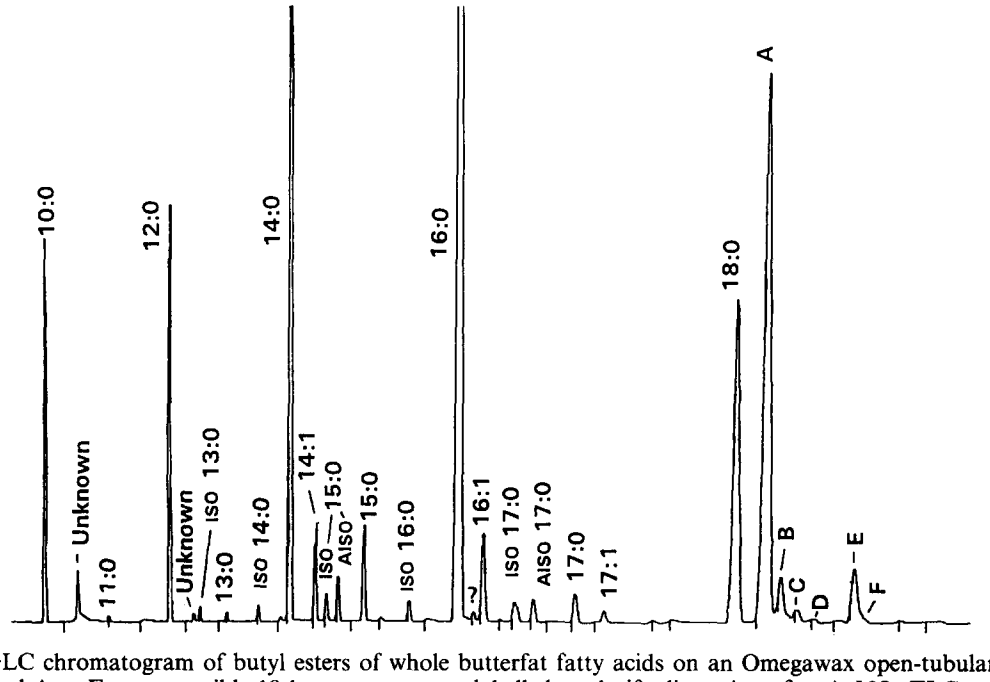

Fig. 1. Part of GLC chromatogram of butyl esters of whole butterfat fatty acids on an Omegawax open-tubular column. Peaks lettered A to F are ostensible 18:1 components, so labelled to clarify discussion after $AgNO_3$-TLC separation.

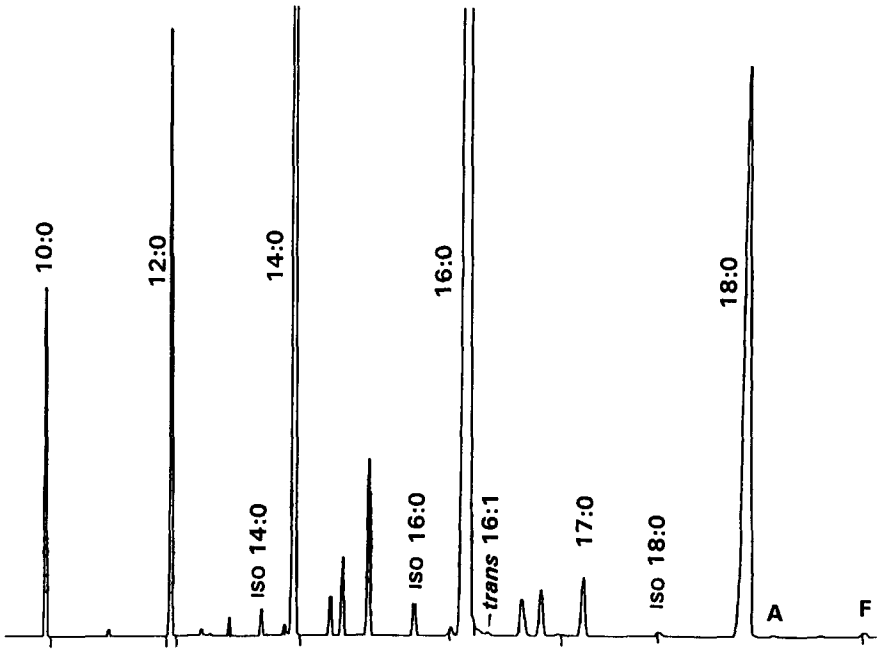

Fig. 2. Part of GLC chromatogram of butyl esters from band 4 of $AgNO_3$-TLC separation of esters of Fig. 1. Conditions as for Fig. 1.

position in linolenic acid. Thus the *trans*-18:1*n*-4 and 18:1*n*-2 isomers are evidently derived from the *cis*-*n*-3-bond in 18:3*n*-3 and *trans*-18:1*n*-3 would not be expected.

$AgNO_3$-TLC is not an exact means of fatty acid isolation (Gunstone *et al.*, 1967; Ackman *et al.*, 1981*a*; Sebedio & Ackman, 1981), since both bond position and chain length affect separations. Other $AgNO_3$-silicic acid separations include conjugated 18:2 isomers which should appear on TLC near the *trans*-monoenes (Hay & Morrison, 1970). However such dienes would appear much later on GLC (Ackman *et al.*, 1981*a*). This band (No. 3) does not include any 14:1, but there are at least three minor *trans*-16:1 peaks. The first is marked and coincides with *cis*-16:1*n*-7 (see band 1 discussions below) and therefore is very probably *trans*-16:1*n*-7. By analogy with the C_{18} isomers there is a high probability that the second is *trans*-16:1*n*-5 and that the third is *trans*-16:1*n*-4. Hay and Morrison (1970) consider that the *trans*-16:1 acids are derived from *trans*-18:1 precursors by chain shortening. The low proportions of both 18:1*n*-6 and 16:1*n*-6 in Fig. 3, for example, would support this view. They report that 20% of 16:1 in their sample was *trans* in geometry and among the 16:1 isomers with bonds remote from the carboxyl group list 32·8% *n*-7, 1·7% *n*-6, 10·6% *n*-5, 12·9% *n*-4, 9·7% *n*-3 and 10·6% *n*-2.

Excluding the *trans*-16:1*n*-3, the proportions reported among the *n*-7, *n*-5 and *n*-4 isomers match the first three small peaks of Fig. 3. The *trans*-16:1*n*-2 is probably the obvious shoulder on the front of the anteiso-

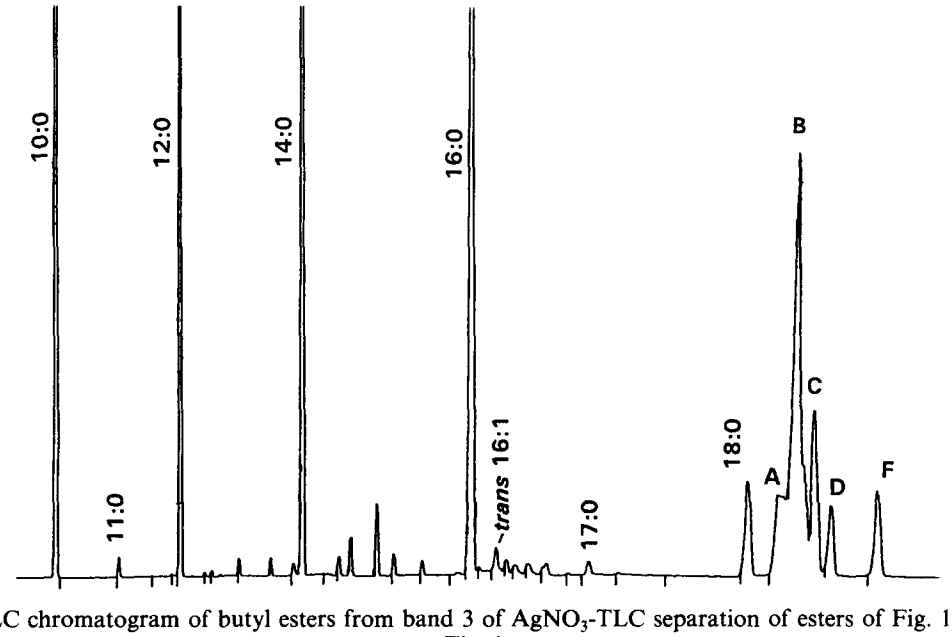

Fig. 3. Part of GLC chromatogram of butyl esters from band 3 of $AgNO_3$-TLC separation of esters of Fig. 1. Conditions as for Fig. 1.

Table 1. Distribution (w/w%) of double bonds in *cis* and *trans* octadecenoic fatty acids from cow butterfats and two cow adipose tissues

Isomer	Position of double bond	Cow[a] milk cream	Cow[b] butter (Av)	Cow adipose tissue[b]	
				Perirenal	Subcutaneous
cis	Δ6 (*n*–12)	—	0·2	0·1	0·2
	7 (*n*–11)	—	0·2	0·2	0·2
	8 (*n*–10)	1·0	0·7	0·5	0·5
	9 (*n*–9)	96·0	94·7	95·3	94·0
	10 (*n*–8)	0·5	0·2	0·2	0·2
	11 (*n*–7)	2·5	3·3	3·1	4·2
	12 (*n*–6)	—	0·4	0·2	0·1
	13 (*n*–5)	—	0·2	0·2	0·5
	14 (*n*–4)	—	0·2	0·2	0·1
trans	Δ6 (*n*–12)	1·0	0·3	0·3	0·2
	7 (*n*–11)	0·8	0·3	0·3	0·3
	7 (*n*–11)	0·8	0·3	0·3	0·3
	8 (*n*–10)	3·2	1·5	1·6	1·5
	9 (*n*–9)	10·2	8·8	8·9	13·6
	10 (*n*–8)	10·5	5·5	5·4	6·4
	11 (*n*–7)	35·7	60·5	68·5	64·4
	12 (*n*–6)	4·1	4·1	2·7	2·4
	13 (*n*–5)	10·5	4·4	2·6	2·3
	14 (*n*–4)	9·0	5·2	3·6	3·6
	15 (*n*–3)	6·8	3·9	2·6	2·3
	16 (*n*–2)	7·5	5·5	3·2	3·0

[a] from Hay & Morrison (1970).
[b] from Parodi (1976).

17:0 peak in this figure. Dietary *trans*-3-hexadecenoic acid (16:1*n*-13), found in green plant photosynthesis lipids, also remains a possibility for one 16:1 component as it does not yet seem to have been followed through the rumen process. It is very mobile on $AgNO_3$-TLC and on GLC on a polar liquid phase it should elute later than most isomers with bonds positionally remote from the carboxyl group, but probably before *trans*-16:1*n*-2 (Gunstone *et al.*, 1967; Ackman, 1972). This is in fact the iso-17:0 position and, as already observed, this peak is skewed by an additional component in the whole butter butyl esters (Fig. 1). The proportion relative to 17:0 in Fig. 3 suggests that the peak is mostly *trans*-16:1*n*-13 with little or no iso-17:0.

Matter (1992), for methyl esters of fatty acids of feta cheese, used DB-wax, a liquid phase very similar to Omegawax. Two samples show a peak after 16:0 and ahead of the main 16:1 peak, but according to Traitler (1987), *cis*- and *trans*-16:1*n*-7 (positional isomer not specified) do not separate on this type of liquid phase. The lesser possible 16:1 component of Matter (1992) is therefore probably not a *trans* isomer but may be identical to the component of unknown structure marked (?) in Fig. 1. A *cis* Δ5 16:1 (16:1*n*-9) would elute well ahead of 16:1*n*-7, based on GLC data for C_{18} (Gunstone *et al.*, 1967) and C_{20} (Wijesundera & Ackman, 1989) fatty acids. This unknown component could also be a C_{16} chain with a simple central-located methyl-branch (17:0) fatty acid of the types described by Ackman *et al.* (1972a) and Duncan and Garton (1978), and thus may match a shoulder on the back of 16:0 in Fig. 2.

Apps & Willemse (1991), on the relatively non-polar 5% phenyl polydimethylsiloxane, show a well-characterized single 16:1 peak from cow milk after the iso-16:0 peak and ahead of the major 16:0 peak, with minor adjacent shoulders. Only about 5% of the total 16:1 area could be *trans*-16:1*n*-7 as on that liquid phase this component should follow *cis*-16:1*n*-7 (Traitler, 1987), but precede 16:0. Their figure shows nothing between 16:0 and iso-17:0, but a single peak closely follows 18:0. Christie (1988) lists *cis*-18:1*n*-2 as following 18:0 on a silicone liquid phase. This is confirmed by Gunstone *et al.*, (1967) who suggest that *trans*-18:1*n*-2 would elute a little earlier (liquid phase Apiezon-L) than the *cis* isomer. GLC on silicones can thus provide an independent peak for the one 18:1*n*-2 isomer, presumably *trans* according to compositions given in Table 1.

The gas-liquid chromatography of the TLC-$AgNO_3$ band 2, nominally all of *cis* esters, includes (Fig. 4) single peaks for 12:1 and 14:1 acids, a single 16:1 peak, evidently *cis*-16:1*n*-7, and a large 18:1 peak, primarily *cis*-18:1*n*-9. The minor 18:1 isomers of Table 1 that have *cis* ethylenic bonds closer to the carboxyl group (Table 1, Jensen *et al.*, 1991; Jensen, 1992) are therefore not resolved from this 'oleic' acid, possibly due to the decrease in resolution resulting from the greater number of total carbons in butyl esters as compared to the usual methyl esters. *Cis*-18:1*n*-8, for example, would not resolve from the major *cis*-18:1*n*-9 peak since butyl esters follow the retention time series set for methyl esters (Christie, 1988). The leading edge of this peak also coincides exactly with that of the corresponding early-eluting *trans* peak A of Fig. 3. The

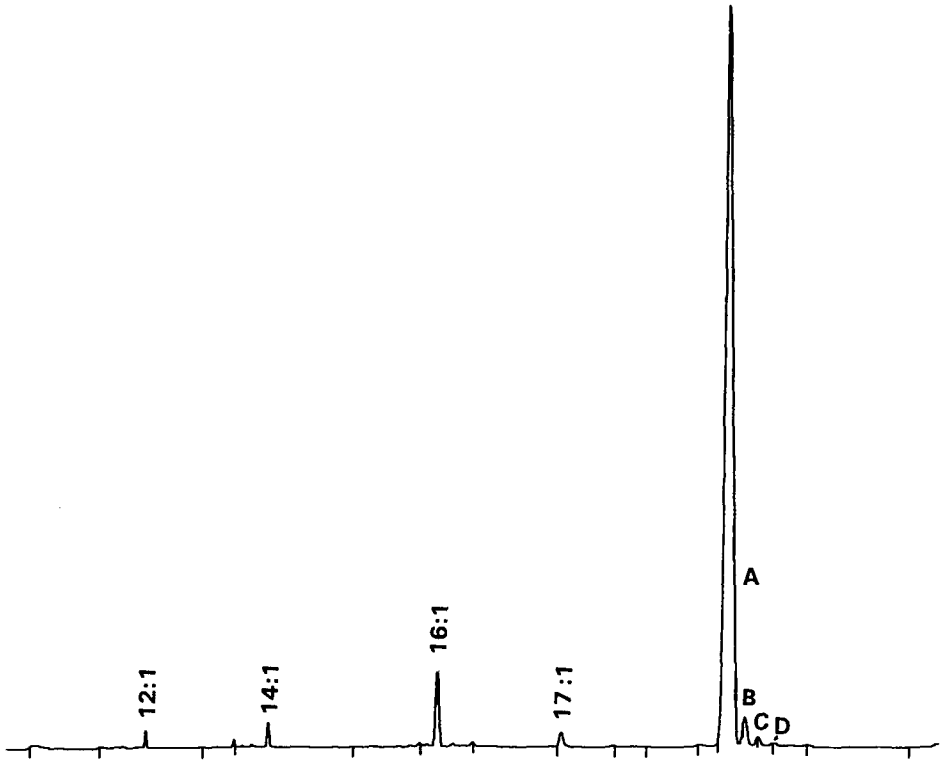

Fig. 4. Part of GLC chromatogram of butyl esters from band 2 of $AgNO_3$-TLC separation of esters of Fig. 1. Conditions as for Fig. 1.

peaks following *cis*-18:1*n*-9 coincide with peaks B,C and D of Figure 3 and are also in the same relative proportions among the three. They are therefore presumed to be *cis*-18:1*n*-7 (*cis*-vaccenic acid), *cis*-18:1*n*-5 and, as suggested by the elution times (Christie, 1988) and the bovine fatty acids of Table 1, *cis*-18:1*n*-4. No peak corresponding to peak F was observed, but in the original butterfat esters this was a trace component, in fact a shoulder on the back of peak E of Fig. 1. Peak E was represented only in band 1 (GLC not shown), the least mobile band on $AgN0_3$-TLC, and is therefore evidently *cis,cis*-18:2*n*-6 (linoleic acid). It was accompanied by several nearby but lesser peaks which were not investigated. The application of $AgNO_3$-TLC to milk fats for separation of polyunsaturated fatty acids is generally recognized as very complex (Murawski & Egge, 1975).

Band 1 contained no 12:1 or 14:1 peaks, but included an important component in the iso-11:0 position, matching the 'unknown' marked in the original esters (Fig. 1). From the $AgNO_3$-TLC position this component is clearly not a saturated fatty acid of any type. As early as 1962 a peak in this region was tentatively identified by Jensen and Sampugna (1962) as a 10:1 fatty acid. The identification of this 'unknown' as a 10:1*n*-1 fatty acid from our work seems certain, and a 10:1 acid has also been quantitated, over the lactation period, in cow milk fat (Renner & Melcher, 1978). The $AgNO_3$-TLC band position indicates that the ethylenic bond is probably not either *cis* or *trans*. The shorter alkyl chain length, and specifically a terminal vinylic bond, would both reduce $AgNO_3$-TLC mobility (Gunstone *et al.*, 1967), so finding such a fatty acid in band 1 is reasonable. The late elution on our GLC column (in the iso-11:0 position) suggests that the bond position is at or near the terminal methyl group (Lie Ken Jie, 1975; Gunstone *et al.*, 1967; Ackman, 1972; Christie, 1988).

Apps and Willemse (1991) have proposed the non-polar liquid phase 5% phenylpolydimethylsiloxane for fast fingerprinting of milks. Their figure supplies some detail agreeing with the various iso and anteiso fatty acids shown in Figs 2 and 3. An appropriate peak for 10:1*n*-1 is shown after iso-10:0 and just ahead of 10:0, matching in quantity the first small 'unknown' component of Figure 1. This peak would also account for the 10:1 fatty acid having a vinyl ethylenic bond since the terminal bond position causes such a fatty acid to appear just before the corresponding saturated acid on a silicone liquid phase (Lie Ken Jie, 1975; Christie, 1988).

A DB-wax capillary column (essentially the same liquid phase as Omegawax) has been applied to identify adulterants in dairy products, meats, etc. (Matter, 1992). Feta cheeses show a peak in the first (cf. Fig. 1) 'unknown' position (that of iso-11:0), a single peak for 14:1 and other details similar to Fig. 1, but the analysis is for methyl esters.

Bitman and Wood (1990) list 10:1 for fatty acids of whole milk fat but not for the phospholipids. The conventional view of action by a Δ9,10 desaturase on saturated acids would give an unusual terminal 10:1 vinyl group, and these vinyl bonds can also be produced by plant desaturases (Ackman, 1964). The 12:1 (marked 'unknown' in Fig. 1) also elutes late, very near the iso-13:0 peak, suggesting a 12:1*n*-3 (*cis*-9-dodecenoate) structure. It can be seen that progressively the 12:1*n*-3, 14:1*n*-5 and 16:1*n*-7 elute earlier and earlier relative to

the corresponding following iso acids, so all facts point to the presence of the *cis* monoethylenic fatty acid series 10:1*n*-1, 12:1*n*-3, 14:1*n*-5 and 16:1*n*-7. These could be produced in the animal tissues proper, as distinct from the rumen. Hay and Morrison (1970) speculate on these options at some length.

In an early application of capillary GLC, the polar liquid phase EGSS-X provided resolution of methyl elaidate (*trans*-18:1*n*-9) and methyl oleate (*cis*-18:1*n*-9), eluting in that order, and of the corresponding *trans*, *trans*- and *cis*,*cis*-9,12-octadecadienoic esters (Lavoue & Bezard, 1969). Traitler (1987) shows the reverse order of elution of 18:1 geometrical isomers on a capillary Carbowax-20M capillary column. The lack of useful resolution of methyl esters of most *trans*-18:1 fatty acids from the corresponding *cis*-18:1 isomers on capillary columns with liquid phases of moderate polarity (e.g. neopentylglycolsuccinate or butanediolsuccinate polyesters) was noted as early as 1967 (Gunstone *et al.*, 1967) and 1972 (Ackman 1972; Ackman *et al.*, 1972*b*). Accordingly, at that time the non-polar liquid phase Apiezon-L was usually preferred for separation of centrally located but geometrically different monoethylenic fatty acids (Gunstone *et al.*, 1967; Ackman *et al.*, 1972b). Subsequently, cyanosilicone liquid phases were introduced (Ackman & Hooper, 1974; Lanza & Slover, 1981; Enig *et al.*, 1983; Slover *et al.*, 1985) and these became the preferred liquid phases for most cases of fatty acid analyses where *cis-trans* resolutions are important (Ratnayake & Beare-Rogers, 1990; Ratnayake *et al.*, 1990*a*; Ratnayake, 1992). The *trans* fatty acids of margarine made from partially hydrogenated vegetable oils have been extensively studied by capillary gas-liquid chromatography (Enig *et al.*, 1983; Mossoba *et al.*, 1990; Ratnayake & Beare-Rogers, 1990; Ratnayake *et al.*, 1990*a*; Ratnayake, 1992). Surprisingly little attention has been paid to similar analyses of butterfat, although *trans* monoethylenic acids have long been known to be 2–5% of the total fatty acids (Sommerfeld, 1983; Craig-Schmidt, 1992; Jensen 1992). Recent evidence that the *trans* monoethylenic fatty acids of butter are innocuous compared to those of margarines (Willett *et al.*, 1993) should stimulate more interest in the *trans* fatty acids of butterfat.

On the liquid phase Carbowax-20M (chemically similar to Omegawax) methyl *trans*-18:1 has been shown to partly separate from methyl *cis*-18:1 (presumably the two are the common n-9 isomers) on a 10 m capillary column (Traitler, 1987), but this brings *trans*-18:1*n*-9 into the *cis*-18:1*n*-7 position. Thus, in the case of methyl esters of butterfat and other ruminant fats, the multiplicity of isomers of both geometries would probably negate the usefulness of such separations. This is hinted at in the feta cheese figures of Matter (1992), with considerable crowding of peaks in the 18:1–18:2 region. For *trans* acid estimation in animal fats sophisticated technology such as 60-m cyanosilicone capillary columns is required (Lin *et al.*, 1984), and even with this length separation results in margarines are incomplete (Ratnayake, 1992). The fall-back position of using $AgNO_3$-TLC as an auxiliary technique was noted by Gunstone *et al.* as early as 1967.

Additional peaks shown by Matter (1992) between 18:1 and 18:2 could be from either the superior resolution of methyl esters compared to butyl esters, or to real or incipient *cis-trans* resolution of minor components. The low proportion of *trans*-18:1n-6 (Fig. 3), is in agreement with the results of Hay and Morrison (1970). The latter also indicate a low proportion of *trans*-16:1*n*-6, which appears to be correct from Fig. 3.

Rumen enzyme specificity provides isomerization of the *cis*-Δ12,13 (*n*-6) bond to a position (Δ11,12) conjugated with the *cis*-Δ9,10 bond, this being a route to *trans*-18:1*n*-7 (Gurr & Harwood, 1991). Similarly, if 18:3*n*-3 (linolenic acid) were in the diet, isomerization of the *cis*-Δ15,16 bond to a conjugated Δ14,15 position could take place, leading eventually to the observed *trans*-18:1*n*-4. The *cis*-Δ15,16 bond of 18:3*n*-3 is known to be very labile (Ratnayake *et al.*, 1990*b*) and its rapid removal by isomerization to *trans*-18:1*n*-2 could explain both the absence of *cis*- and *trans*-18:1*n*-3 and the eventual formation of *trans*-18:1*n*-2 by reduction of the other two ethylenic bonds of the 18:3*n*-3 molecule. The *trans*-18:2*n*-2 must surely be formed from 18:3*n*-3 and not from 18:2*n*-6, and the proportion of these two acids ingested from feedstuffs, or forage may explain some differences in butter fatty acids. The effect of winter and summer grazing conditions is a well-known phenomenon (Renner & Melcher, 1978), and the effect on the consistency of South African butter is mentioned by Ackman and Hooper (1973). The suggestion of Hay and Morrison (1970) that β-oxidation of *trans* 18:1 acids leads to some *trans* 16:1 acids seems a reasonable explanation for the *trans* 16:1*n*-7, *n*-6 and other 16:1 isomer proportions. The 10:1–12:1–14:1 series of *cis* isomers with Δ9,10 bonds seem to have a unique and separate non-rumen origin, but could overlap with the chain-shortening at 16:1 since both *cis* *n*-9 and *n*-5 isomers are present along with the major 16:1*n*-7 isomer (Hay & Morrison, 1970). It should be noted that part of Table 1 is for fatty acids of bovine depot fat, not butterfat. It is possible that overoxidation (Ackman *et al.*, 1981*b*) during identification of bond positions by cleavage created products matching the extra *cis* or *trans* isomers, e.g. 14:1*n*-9, *n*-8, *n*-7, *n*-6) of Hay and Morrison (1970). The oddity of our analyses is the absence of any type of 18:1*n*-3. This did not come to our notice in any GLC analyses of one butterfat sample.

Overall, the butyl esters of butterfat can be readily analysed in detail from 4:0 to 18:2*n*-6 in 30 min or less by open-tubular gas-liquid chromatography with a polyglycol-type liquid phase in a 30 m column. The complete coincidence of the butyl esters of common *cis*- and *trans*-18:1 fatty acids simplifies interpretation and reduces data output demands that would be created by additional peaks from *cis-trans* separations on cyanosilicone liquid phases.

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