Distribution Modes and Possible Origins of Sheep Wool Hydrocarbons

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Abstract

An unusual mode of distribution of low-molecular-weight n-alkanes (C_{11}–C_{15}) with a slight even carbon-number predominance has been observed in the gas chromatographic analysis of the paraffin fraction which was extracted from the wool of live sheep. This is in addition to the other two modes of medium-, C_{15}–C_{24}, and high-, C_{24}–C_{33}, molecular-weight alkanes usually present in wool wax which have also been found in sheep's manure. The high-molecular-weight hydrocarbons (predominantly odd carbon-numbered) are presumed to be derived from the diet (pasture plants) whereas the other two hydrocarbon distributions probably result from the combined action of animal and microbial metabolism.

Introduction

THE GAS CHROMATOGRAPHIC ANALYSIS of the normal paraffins in wool wax was first reported by Downing et al. (1). Recently the paraffinic hydrocarbons of wool wax have been characterized in detail by the investigations of Mold et al. (2,3). An interesting observation of these studies is the presence of two distinct distribution groups or modes of normal paraffins: a low-molecular-weight mode in the C_{13}–C_{25} range, and a second mode of about C_{23}–C_{35} carbon atoms with a pronounced odd-to-even ratio.

By the use of a combination gas chromatograph-mass spectrometer of the type described by Ryhage (4), a number of n-paraffin distributions which were obtained from natural products have been analyzed in this laboratory (5–8). As a part of this general program the normal paraffins isolated from sheep's wool have also been examined. The approach has been to analyze wool collected from live sheep in an attempt to characterize the natural distribution of wool hydrocarbons as they exist in the animal. This is in contrast to the work of the previous investigators where samples of centrifugal wool grease or hydrolyzed wool wax were used.

In addition to the two families of homologous alkanes previously reported, a third distribution group of low-molecular-weight hydrocarbons has been observed in the range C_{11}–C_{15}. The interpretation of the wool wax hydrocarbons has been considered in terms of the various factors which might give rise to such relatively complex distribution, namely, products of sheep metabolism, metabolic end-products of enteric bacteria, unaltered products of dietary origin, and end-products of aerobic bacterial degradation.

Experimental Section

Samples of wool were taken from live adult sheep in two different locations. One sample of wool was obtained from sheep pastured on the ranch of J. O. Bear, Lampasas, Tex. (Sample I); the other sample was collected from two sheep in the Port City Stockyards, Houston, Tex. (Sample II). It was also noticed that wool taken from live sheep was invariably contaminated with both feces and soil such that the outermost layer was dirty and matted.

In the case of Sample II this outer layer was separated from the considerably whiter wool which lay closest to the skin, and the two fractions were analyzed independently. Wool Sample I however was analyzed without prior separation. Ten-gram aliquots of the wool were placed on an all-glass Soxhlet apparatus (previously cleaned with hot chromic acid) and extracted with 50 ml of benzene:methanol (3:1) for 12 hours (9). The extract was subsequently transferred to a 100-ml round-bottomed flask and concentrated to dryness by use of a rotary vacuum pump. The residue was transferred to a heptane-washed silica gel column (1 cm by 16 cm) by means of two 5-ml volumes of *n*-heptane. The silica gel had previously been activated by heating at 400C in a furnace for 10 hr.

The column was eluted with 20 ml of *n*-heptane and, after slow concentration at ambient temperature to a suitable volume in a stream of dry nitrogen, a 1.0 μl sample of this residue was injected into the gas chromatograph with a 10 μl Hamilton syringe. Analyses were performed on a 60 meter × 0.5 mm I.D. stainless steel capillary column, coated with 10% Apiezon L by the use of a Barber-Colman Model 1520 flame ionization gas chromatograph. Mass spectra were taken with the LKB combination gas chromatograph-mass spectrometer (LKB Producter, Bromma I, Sweden).

Results and Discussion

The distribution of paraffinic hydrocarbons isolated from wool wax (Sample II) is illustrated in Figure 1. The relative amounts of normal paraffins from Samples I and II are given in Table I. There are significant quantitative differences between these two samples; however they are qualitatively similar. In Figure 1 the upper chromatogram A is from the outermost contaminated wool layer whereas the lower trace B represents the wool which lay closest to the skin. There are subtle differences between these two samples; most obvious is the larger number of unresolved isomeric peaks in A. Less obvious is an even-to-odd ratio for the C_{11}–C_{15} alkanes in 1A yet an odd-to-even ratio for the same distribution in 1B; furthermore the later chromatogram has slightly greater relative amounts of n-nonacosane and n-hentriacontane. The major peaks were all found to have mass spectral patterns consistent with n-alkanes. Two representative mass spectra of peaks C_{13} and C_{14} (taken from Figure 1B) are shown in Figure 2 and are typically those to be expected from normal paraffins. Peaks A and B in Figure 1 have been identified by mass spectrometry to be pristane and phytane respectively, which confirms the earlier report by Mold et al. (1). The low-molecular-weight alkanes in the C_{11}–C_{15} range have not been previously observed. They are however of sufficiently low molecular weight to be lost in many commonly employed extraction proce-

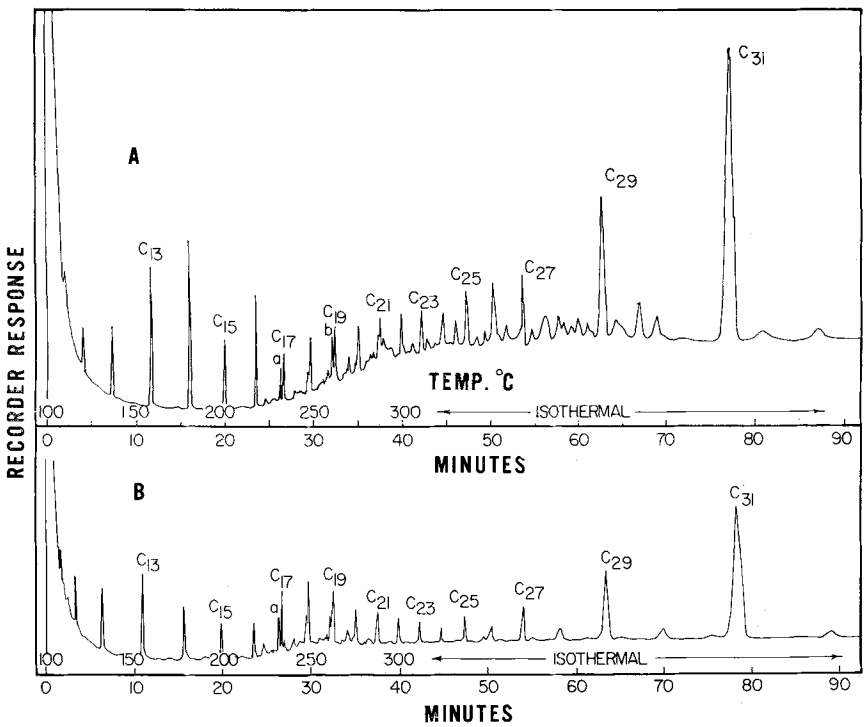

FIG. 1. Gas chromatographic separation of hydrocarbons of sheep wool on a 60-m × 0.5-mm stainless steel tubing coated with 10% Apiezon L. (A) Outer portion of wool (B) Inner portion of wool (closer to skin).

dures. In the authors' experience, sample loss appears to be dependent on the temperature at which sample concentration takes place.

In the C_{25}–C_{32} range the carbon preference index (CPI) or ratio of the relative peak areas (p.a.) ratio Σp.a. odd C_{25}–C_{31}/Σp.a. even C_{26}–C_{32} have a value of 5.17 for Sample I and a value of 5.09 for Sample II.

A similar odd-even ratio has been obtained from the analysis of alkanes isolated from sheep manure. From comparable calculations between paraffins isolated from cattle manure (4.6; 5.5) and those extracted from pasture plants (4.9; 5.3) it has been demonstrated that the higher-molecular-weight alkanes originate almost exclusively from the diet (5,6). Similar arguments may also be applied to explain the origin of the C_{24}–C_{33} alkanes in sheep wool and manure.

The C_{15}–C_{24} distribution of paraffins observed in sheep wool is also qualitatively represented in the sheep manure; moreover the odd-to-even ratio is small or approaches unity. It is perhaps significant that similar distributions of n-alkanes with comparable odd-even ratios have been found in some microorganisms (10). The hypothesis that the C_{15}–C_{24} alkanes are products of enteric bacterial metabolism is an intriguing one and certainly merits consideration, together with the possibilities that these hydrocarbons are end-products of either host epidermal or skin bacterial metabolism.

The lowest-molecular-weight hydrocarbons in the C_{11}–C_{15} range are particularly interesting but should be evaluated with caution since, as previously indi-

TABLE I

Relative Percentage Amounts of Sheep Wool *n*-Alkanes[a]

	Sample I	Sample II
n-C_{11}	2.2	0.53
n-C_{12}	4.5	0.66
n-C_{13}	7.1	1.0
n-C_{14}	4.5	1.3
n-C_{15}	2.8	1.2
n-C_{16}	2.8	2.1
n-C_{17}	4.2	3.6
n-C_{18}	4.7	4.0
n-C_{19}	3.9	4.0
n-C_{20}	2.4	2.8
n-C_{21}	1.8	2.9
n-C_{22}	1.7	3.0
n-C_{23}	1.3	4.5
n-C_{24}	1.1	2.0
n-C_{25}	1.9	4.6
n-C_{26}	1.8	2.1
n-C_{27}	3.5	8.5
n-C_{28}	1.9	2.0
n-C_{29}	10.2	21.7
n-C_{30}	2.8	2.9
n-C_{31}	30.5	20.7
n-C_{32}	2.4	3.9

[a] Values calculated by triangulation of chromatogram peak areas.

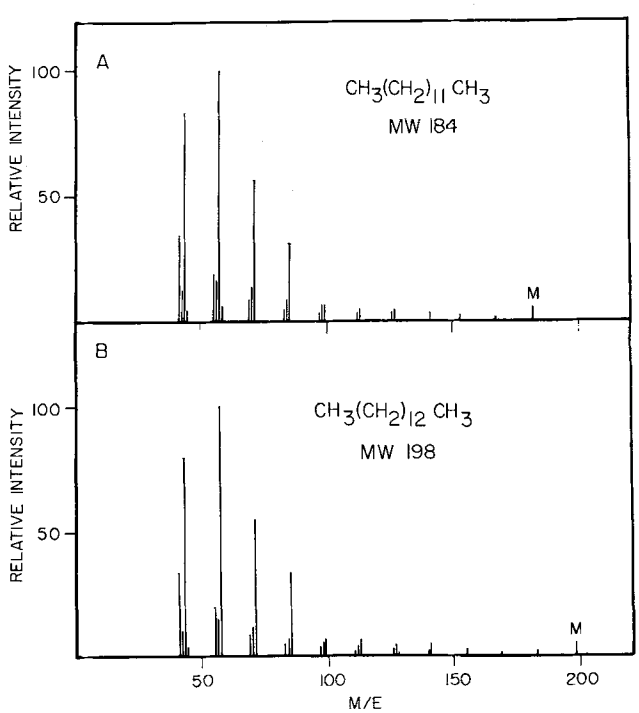

FIG. 2. A. Mass spectrum of peak C_{13} in Figure 1. B. Mass spectrum of peak C_{14} in Figure 1.

cated, these paraffins are most easily lost by evaporation. While it is well established that most ruminants have profuse intestinal flora, it should also be remembered that the fleece itself supports active flora. Maxwell (11) in a careful study has isolated 83 different species of aerobic bacteria from sheep's wool; the most prominent members isolated were Pseudomonas, *Proteus vulgaris,* and Achromobacter. It is conceivable that these and other facultative aerobes may modify the alkane distribution in some way, either by production of low-molecular-weight hydrocarbons directly or through the degradation of existing metabolites. If this is true, then the even-to-odd distribution of these hydrocarbons in the outer, more contaminated wool layers (Figure 1A) could be explained by microbial oxidation of preformed predominantly odd carbon-numbered hydrocarbons into fatty acids and subsequent decarboxylation of the resulting fatty acids into hydrocarbons. A less likely alternative explanation could be the reductive conversion of low-molecular-weight, predominantly even carbon-numbered, fatty acids to alkanes by anaerobic microorganisms. Experiments with radioactively labelled substrates and microbial cultures along the lines of similar work carried out in this laboratory (12–14) should provide definite answers to these questions.

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