

A STUDY OF LIPIDS FROM
ANIMAL SKIN SECRETIONS

A thesis
presented to the University of Cape Town
for the degree of
Doctor of Philosophy

by

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Pretoria.

February, 1955.

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INTRODUCTION

Within the skin of animals are situated various glands which secrete their products to the outer surface of the skin. The glands exist in many forms and their functions are diverse. A satisfactory classification based on the morphology of the glands has been worked out and the glands of the vertebrata are recognised as belonging to three main types :- holocrine, eccrine and apocrine glands. In man the holocrine glands are referred to as sebaceous and the eccrine as sudoriferous or sweat glands. These terms are often used also for animals.

Comparatively few animal skin glands or secretions have been investigated chemically. The sebaceous secretion of the sheep, wool wax, has been much investigated owing to the commercial value of this product; and the author participated for some years in studies of wool wax at the National Chemical Research Laboratory, Pretoria. In Chapter I is summarised the present knowledge of wool wax chemistry, including contributions from this laboratory into the investigation of dihydric alcohols and hydroxy-acids and the syntheses of iso-acids which occur in wool wax.

Skin secretions which have no commercial applications are nevertheless of great interest from a physiological and biochemical point of view. In this respect human skin secretions may naturally attract more attention than those of other animals. Their investigation may prove rewarding in throwing further light upon the studies of dermatological disorders in general, and more particularly upon the all important problem of skin cancer. A study of human sebaceous secretions was taken up in this laboratory by the author; the aliphatic alcohols and some polycyclic hydrocarbons present were investigated. The chemistry of human sebaceous secretions is reviewed in Chapter II.

Chapter III reviews work of other investigators on various skin gland secretions. The "scent" glands of musk deer and other animals and the "preen" glands of some birds have been investigated. Some materials of human origin such as ear wax, which are considered as related to sebaceous secretions, are included in this review.

Natural fats and waxes are generally of complex composition and early workers did not succeed in isolating many pure components from such materials. As a result of improved techniques and methods considerable progress has been made in the last few years towards the elucidation of the fine structures of fats and waxes.

CHAPTER I

WOOL WAX

A review including the present advances.

Wool wax or lanolin is the fat-soluble substance found in sheep wool; it originates largely from the sebaceous glands. The water-soluble material, suint, which also accumulates in the wool is largely a product of the sudoriferous glands.

Raw wool contains on an average some 15 per cent of wool wax. The wax is of necessity removed before the wool is utilised for spinning, and the wax can therefore be recovered in large quantities as a by-product from the wool industry. Its commercial value lies mainly in its uses for pharmaceutical and veterinary preparations, but there are also many other applications of the wax (1).

Chemical investigations of wool wax started about a hundred years ago. The wax was early established as consisting of fatty acids esterified with cholesterol and other alcohols, glycerol excluded. A small amount of free fatty acids is also present. The so-called "ischolesterol" was early recognised, but this substance is now known to be a mixture of triterpene alcohols. The early literature has been reviewed by Gillespie (2) and Truter (3).

Most investigators have saponified the wax and studied separately the acid and neutral fractions so obtained. Truter (4) has attempted the isolation of individual esters from the unsaponified wax. He claims to have found cholesteryl esters of C_{27} , C_{29} and C_{31} anteiso-acids * and esters of a high molecular weight hydroxy-acid.

The more recent contributions to the chemistry of wool wax are reviewed below. The unsaponifiable and the fatty acid fractions, which each constitute about 50 per cent of the wax, are described separately.

* anteiso- denotes a terminal sec-butyl group.

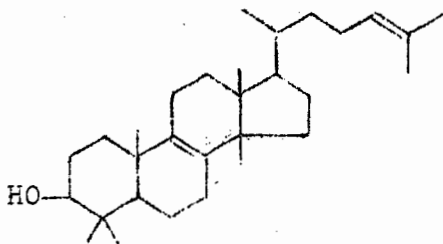
THE UNSAPONIFIABLE MATTER OF WOOL WAX.

Four groups of compounds are recognised in the unsaponifiable fraction : - sterols, triterpene alcohols, aliphatic mono- and dihydric alcohols and hydrocarbons.

The hydrocarbon fraction is negligible in amount (5); its composition has not been reported upon.

The sterol group of which cholesterol is the characteristic compound constitutes about 30 per cent of the unsaponifiable matter. Other sterols derived from cholesterol are present in smaller amounts, such as dihydrocholesterol (6) and cholesta-3:5-dien-7-one. The latter compound is an artefact which is produced in wool wax on saponification with hot alcoholic potassium hydroxide (5).

The triterpene group which constitutes about 25 per cent of the unsaponifiable matter contains the four closely related alcohols lanosterol, dihydrolanosterol, agnosterol and dihydroagnosterol (7). Experiments have indicated that lanosterol is synthesised in the skin (8). It is not certain whether agnosterol (dehydrolanosterol) is a biological product or an artefact (3). After prolonged research mainly by Swiss, British and Australian workers the structure of lanosterol (I) has now been elucidated. The total synthesis of dihydrolanosterol has confirmed the structure (9). The molecule is a peculiar hybrid between sterol and triterpene, with a ring skeleton and side chain like cholesterol and with three extra methyl groups substituted in the rings. Lanosterol occurs also in butter fat (10), mussel fat (11) and the latex of Euphorbia balsamifera (12).



(I)

The aliphatic alcohols constitute 20 to 25 per cent of the unsaponifiable matter and consist of a complex mixture of normal, branched and dihydric alcohols. A critical review of the aliphatic alcohols has been written by Knol (13). By fractional distillation of the acetates, Murray and Schoenfeld (14) succeeded in isolating ten branched chain alcohols, viz : - the iso-alcohols C_{20} , C_{22} , C_{24} and C_{26} and the anteiso-alcohols C_{17} , C_{19} , C_{21} , C_{23} , C_{25} and C_{27} . No normal alcohols were detected. Later, however, Murray isolated small amounts of the seven normal alcohols C_{18} , C_{20} , C_{22} , C_{24} , C_{26} , C_{28} and C_{30} by a special adaptation of the method of urea complex formation; this work has not yet been published (cf. 13). Truter (15) has reported the isolation of the five normal alcohols C_{18} , C_{20} , C_{22} , C_{24} and C_{26} ; they were isolated from the unsaponifiable matter by urea complex formation and further separated into individual components by fractional crystallisation of the urea complexes. Although the alcohols were not pure as was evident from their analytical data, the fact that fairly correct acetyl values and melting points were obtained, excludes the possibility of Truter's products having been branched chain alcohols. Unsaturated aliphatic alcohols have not been detected in wool wax.

The presence of a dihydric alcohol in wool wax was indicated by Kuwata and Katuno (16) who isolated a product with the proposed formula $C_{21}H_{40}(OH)_2$. Horn and the writer (17) obtained a small fraction of dihydric alcohols by chromatography of the unsaponifiable matter of commercial wool wax. The alcohols were identified as 1:2-diols on the grounds that they yielded formaldehyde on oxidation with periodic acid or lead tetra-acetate. In order to avoid possible contamination with scouring chemicals or machine oils the dihydric alcohols were later isolated from wool wax obtained by solvent extraction of merino wool. The wax was saponified under mild conditions in order to prevent formation of artefacts. The unsaponifiable matter was extracted with hexane from a solution of the saponified wax in alkaline aqueous alcohol. By chromatography of the unsaponifiable matter on activated alumina, dihydric alcohols were obtained in a yield of 4 to 5 per cent.

These were fractionated by distillation of the acetates. By saponification of the appropriate distillates and crystallisation, five pure diols were obtained. They were identified as n-hexadecane-, 16-methylheptadecane-, 18-methylnonadecane-, 20-methylheneicosane- and 22-methyltricosane-1:2-diols, by oxidation with lead tetraacetate to the corresponding fatty acids having one methylene group less than the parent diols. The acids produced were compared with synthetically prepared acids of known structure (18).

The author's contribution to this work was the extraction of wax from wool, saponification of the wax, isolation of the unsaponifiable matter and isolation of the glycols by chromatography; in addition two of the iso-acids were synthesised (cf. "The acids of wool wax").

THE ACIDS OF WOOL WAX.

(a) Natural acids.

The acidic fraction of wool wax is not less complex than the neutral fraction. Much confusion existed in the literature on this subject until the field was extensively cleared up by Weitkamp's (19) investigation of the "combined" * acids of wool wax. Recent improvements in column packings allowed him to carry out an efficient fractionation of the acids by vacuum distillation of the methyl esters. Fractionation of small quantities was perfected with a hydrocarbon diluent. Weitkamp isolated thirty-two acids which were identified as belonging to four homologous series as follows :

nine normal acids, even numbered :	C_{10} to C_{26}
ten <u>iso</u> -acids, even numbered :	C_{10} to C_{28}
eleven <u>anteiso</u> -acids, odd numbered :	C_9 to C_{27} and C_{31}
two hydroxy-acids :	C_{14} and C_{16}

The structures of the iso- and anteiso-acids were elucidated by a novel method based on the observation that the solidification

* "combined" denotes acids released from combination by saponification.

point curves for binary mixtures of branched and normal chain acids exhibited two or one transitions respectively, according to whether the length of the normal acid was equal to or longer than the unbranched portion of the branched acid.

Some 60 per cent of the total acids of wool wax were accounted for in Weitkamp's work. The individual acids were present in amounts varying from 0.1 to 5 per cent.

The hydroxy-acids isolated by Weitkamp amounted to less than 4 per cent of the total acids. Kuwata has isolated 2-hydroxyhexadecanoic acid from wool wax acids in 7 per cent yield (20). Bertram (21) inferred from analytical data that wool wax contained hydroxy-acids in a considerably larger proportion than was indicated by these authors. Further, his assumption was not contradicted by the fact that Weitkamp succeeded in isolating only a small amount of hydroxy-acids as some 12 per cent of Weitkamp's acids was not recovered from an initial chromatogram and a further 6 per cent was lost in the distillation. It may be pointed out that it is likely that the more polar and thermolabile hydroxy-acids of wool wax were preferentially included in these losses.

A reinvestigation of the hydroxy-acids was therefore taken up in this laboratory (22). The hydroxylated acids were separated in good yield from the unhydroxylated acids by the Craig solvent distribution method, which also sufficed for the isolation of some of the pure single components. Some 28 per cent of the total acids was identified as 2-hydroxy-n-dodecanoic, 2-hydroxy-n-tetradecanoic, 2-hydroxy-n-hexadecanoic acids and 2-hydroxyoctadecanoic acid isomers (23).

These acids were also characterised as the corresponding dihydric alcohols (24) : the methyl esters of the total wool wax acids were reduced with lithium aluminium hydride and the 1:2-diols originating from the α -hydroxy-acids were separated by chromatography. Distillation of the acetates and hydrolysis and crystallisation of appropriate fractions yielded pure n-tetradecane-, n-hexadecane-,

n-octadecane- and 16-methylheptadecane-1:2-diols. The unresolved C_{18} α -hydroxy-acid isomers were thus characterised as 2-hydroxy-n-octadecanoic and 2-hydroxy-16-methylheptadecanoic acids.

The author's contribution to this work was the isolation of the total acids from wool wax, preparation of the methyl esters, reduction of the esters with lithium aluminium hydride and isolation of the diols by chromatography.

American workers (25) have recently claimed the isolation from wool wax of a δ -lactone (?), or a mixture of lactones, with the approximate composition $C_{20}H_{38}O_2$. The size of the lactone ring was inferred from infra-red absorption data which, however, do not exclude the possibility of a structure having more than six atoms in the ring (26).

(b) Synthetic acids.

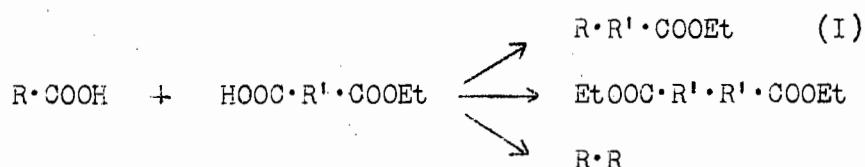
Branched chain acids are relatively rare in nature and long chain iso- and anteiso-acids had not been encountered before Weitkamp discovered their presence in wool wax. Following Anderson's (27) important discovery of biologically active branched chain acids in the tubercle bacillus, the investigation of branched chain acids has been extensively pursued by many workers. Improved methods of synthesis have been developed and a great variety of branched chain acids has been synthesised.

Five of the naturally occurring iso-acids of wool wax were synthesised in this laboratory (28). The members containing 10, 12, 14, 18 and 20 carbon atoms were synthesised and the acids and amides characterised. The main purpose of the syntheses was to make exact comparisons between the properties of the synthetic iso-acids and those isolated by Weitkamp, since Weitkamp's method of assigning structures to his series was novel and few direct comparisons had been made. The discrepancy between the melting point of the 12-methyltridecanoic acid synthesised by Fordyce and Johnson (29) and that of the natural specimen isolated from wool wax by Weitkamp was cleared up in favour of the

latter by the synthesis of this acid. Of four modern methods of chain lengthening which were employed in these syntheses the anodic method as used by Linstead (30) was found superior as regards yield and purity of the products and simplicity of operation.

A further purpose of the syntheses was to provide a series of iso-compounds to serve as reference substances for comparison with degradation products from the hydroxy-acids and 1:2-glycols of wool wax. Three iso-acids which were required for this purpose were also synthesised (18, 24). They were the members containing 17, 21 and 23 carbon atoms.

Of the eight iso-acids, the author synthesised 10-methylundecanoic, 18-methylnonadecanoic, 19-methyleicosanoic and 21-methyldocosanoic acid, none of which had previously been synthesised. Three of the acids were synthesised by the anodic method (30), in which a monocarboxylic acid and the half ester of a dicarboxylic acid in methanolic solution are electrolysed between platinum electrodes to yield a monocarboxylic acid, a dicarboxylic acid and a hydrocarbon.



The desired reaction product (I) was isolated from the unwanted dicarboxylic acid by distillation in vacuum; it was shown that this separation was easily effected also by Craig's counter-current distribution method. 10-Methylundecanoic acid was synthesised from iso-valeric acid and ethyl hydrogen azelate; 18-methylnonadecanoic acid from 10-methylundecanoic acid and ethyl hydrogen sebacate and 21-methyldocosanoic acid from 18-methylnonadecanoic acid and ethyl hydrogen glutarate. The latter half ester was prepared from glutaric acid (31) which was synthesised from acrylonitrile and sodio-malonic ester (32). 19-Methyleicosanoic acid was synthesised from 18-methylnonadecanoic acid via the alcohol, iodide and nitrile (33).

Direct comparison of the synthetic C_{12} and C_{20} iso-acids with specimens isolated by Dr. Weitkamp from wool wax showed the synthetic and natural acids to be identical and thereby afforded further confirmation of the validity of Weitkamp's structural assignments in the iso-acid series.

Milburn and Truter (34) have recently reported the syntheses by the anodic method of iso- and anteiso- acids occurring naturally in wool wax.

CHAPTER II

HUMAN SEBUM

Human skin contains sweat, apocrine and sebaceous glands.

The sweat glands are present in all parts of the skin with a few exceptions such as the lips, eyelids etc. Their minute size is illustrated by the fact that the glands in some areas number more than a thousand per square centimetre of skin. Each gland has its separate duct which winds through the epidermis and opens upon the skin surface. In a man at rest, sweat is secreted in an average amount of one litre per day. Physical exercise can cause a fifty to seventy-five times increase in the normal rate of sweating. The most important function of the sweat glands is their aid in regulation of the body temperature. The glands are also organs for excretion of metabolic waste products although this function is not considered significant.

The chemistry of sweat is comparatively simple and its approximate overall composition has long been known. It contains 99 per cent water, half a per cent or less of sodium chloride and traces of various other constituents such as potassium, calcium, magnesium, copper, manganese, iron, sulphates, phosphates, ammonia, urea, uric acid, creatinine, amino acids, glucose, lactic acid, water-soluble vitamins, phenol and histamine (35). A much disputed question is whether sweat glands also secrete lipid matter (36).

The apocrine glands are restricted to certain areas such as the axillary region (armpit). Their ducts open into the hair follicles. The apocrine secretion has some resemblance to sweat and is often referred to as apocrine "sweat". Its physiology and chemistry is little known. The secretion does not appear on thermal stimulation like eccrine sweat but on emotional stimuli such as stress, pain, fright and excitement (37). Nitta and Ikai (38) have investigated the volatile fatty acids of axillary "sweat" by paper

chromatography and found acids having 2, 3, 4, 5 and 6 carbon atoms.

The sebaceous glands, like sweat glands, are distributed over most of the body surface. They are absent in the palms of hands and soles of feet. The glands are mostly situated around the hair follicles into which the secretion is expelled. Only in a few hairless areas such as the lips, eyelids etc. do the ducts open upon the free surface of the skin.

The sebaceous secretion is called sebum. Several workers have been engaged in the difficult task of measuring the rate of sebaceous secretion from various parts of the body. The total secretion from the body is probably less than one gram of sebum per 24 hours (39). The full significance of sebum is not understood. It gives pliability to the skin and thereby also prevents the formation of cracks or fissures through which micro-organisms can penetrate. It also protects against the disintegrating influence of external moisture. The sebaceous secretion participates, although to a lesser degree than sweat, in regulation of the body temperature. It is not known whether metabolic waste products are excreted via sebaceous glands.

The chemistry of sebum has been investigated by many during the last seventy years. The earliest literature is reviewed by Unna and Golodetz (40) and later contributions by Čmelik (41). The early workers found sebum to consist of glycerides and wax esters. Cholesterol was also early recognised as a component. The composition of sebum varies considerably as indicated by the following figures of Wheatley (42):

Free fatty acids	22 - 32%
"Combined" acids	28 - 41%
Unsaponifiable matter	27 - 36%

Before entering into a description of sebum in detail, it is appropriate to discuss the general difficulty that exists in assessing the true origin of the materials used by most investigators. For practical reasons most investigators have collected surface skin

"fat" rather than pure sebum as obtained from inside the sebaceous ducts. Although surface skin "fat" undoubtedly arises mainly from sebaceous glands, it may in part originate from other sources : sweat glands, which are present in virtually all areas of the skin, may secrete lipid matter. It is also believed that fats from the cells of the epidermis diffuse out to the skin surface (43). Conversely, it is possible that sebaceous secretions contain water-soluble matter which in that case would be incorrectly included by investigators as part of the sweat. Some recent investigations by Schmidt-Nielson (44) were carried out on sebum obtained from individual sebaceous orifices.

The chemistry of the acid and unsaponifiable fractions of sebum is reviewed below and then follows the author's contribution in this field.

A REVIEW OF PREVIOUS WORK.

(a) The acids of sebum.

A major contribution to the chemistry of sebum was an investigation of the free fatty acids by Weitkamp, Smiljanic and Rothman (45). By amplified distillation of the methyl esters of the free fatty acids, followed by low temperature crystallisation of individual fractions for the separation of saturated and unsaturated compounds, the following normal chain acids were isolated and identified :

Fourteen saturated acids,
odd and even numbered : C_7 to C_{18} , C_{20} and C_{22}

Eleven mono-unsaturated acids with double bonds
in positions as indicated in brackets :

$C_{11}(2,3?)$, $C_{12}(3,4?)$, $C_{13}(4,5?)$, $C_{14}(5,6)$,
 $C_{15}(6,7)$, $C_{16}(6,7)$, $C_{17}(6,7)$, $C_{17}(8,9)$,
 $C_{18}(6,7)$, $C_{18}(8,9)$, $C_{18}(9,10)$

Acids with more than one double bond : the C_{18}
mixture contained dienoic and trienoic acids¹⁸
with double bonds apparently in unusual positions.

The total fatty acids of sebum have recently been studied by Miescher, Lincke and Rinderknecht (46) who fractionated the methyl esters by distillation. Owing to the small amount of material distilled the longer chain acids were not obtained in the distillates. No pure acids were isolated; the distilled fractions were examined by their melting points, hydrogenation values and the melting points of the hydrogenated products. The calculated composition of the total acid fraction is tabulated below. The results of Weitkamp are included for comparison :

Chain length	TOTAL ACIDS (Miescher)		FREE ACIDS (Weitkamp)	
	Composition (%)	Average number of double bonds	Composition (%)	Average number of double bonds
< C ₁₄	6	0.8	5.8	0.4
C ₁₄ -C ₁₅	22-29	0.25-0.3	15.5	0.15-0.25
C ₁₆	25-40	0.5	36	0.5
> C ₁₆	32 (residue)		39	

The lower molecular weight fraction of the total acids is considerably more unsaturated than the corresponding fraction of the free fatty acids. The overall composition is otherwise similar for the total and the free fatty acids.

Longer chain normal fatty acids having odd numbers of carbon atoms had not been encountered in natural products before Weitkamp isolated several from sebum. Many earlier investigators have claimed the isolation of odd numbered acids from various sources; subsequent work, however, has invariably revealed that such products were mixtures of even numbered acids (47). Recently Shorland and co-workers (47) have isolated several odd numbered acids in very small amounts from natural products. n-Pentadecanoic acid was isolated from butter fat and from hydrogenated mutton tallow and shark liver oil and n-heptadecanoic acid from hydrogenated mutton tallow. The consecutive

series of volatile fatty acids C_2 to C_{10} , including the odd numbered acids, was identified in hydrogenated ox perinephric fat.

The fungistatic and bactericidal properties of the acids of sebum are of interest in connection with self-sterilising properties of the skin. Rothman et al. (48) found that sebum of adults but not sebum of children was fungistatic to the fungus Microsporon audouini which is the cause of the commonly occurring disease "ringworm" of the scalp. Fractionation of active sebum revealed that the fungistatic properties were confined to the free fatty acid fraction and more particularly to the odd numbered acids C_7 to C_{13} of this fraction. The immunity of adults to "ringworm" was explained by the fact that sebum of adults contained the mentioned fungistatic odd numbered acids in five times the amount that was present in sebum of children.

A later investigation by Kligman and Ginsberg (49) has cast some doubt upon the validity of Rothman's explanation for the immunity of the adult scalp against "ringworm". Kligman and Ginsberg found that the sebums of adults and children were similar in fungistatic activity when tested on M. audouini. The fungus was also grown in vitro on hair from adults and children, before and after removal of the natural hair grease by extraction; no significant difference was found between the hair of children and adults in this experiment and the fungus grew equally on hair that contained sebum and hair that had been freed from sebum by extraction.

Miescher et al. (46) tested the bactericidal activity of sebum on Streptococcus haemolyticus, Escherichia coli and Staphylococcus aureus and found that not only the free fatty acid fraction was active but also the neutral fraction of sebum. Of the total fatty acids the fractions of lower molecular weight were the more active. Most fractions showed increased bactericidal activity after long storage, apparently because products of autoxidation were then present.

(b) The unsaponifiable matter of sebum.

By chromatographic studies MacKenna, Wheatley and Wormald (50)

found the unsaponifiable matter of sebum to consist chiefly of hydrocarbons, aliphatic alcohols and cholesterol. Lanosterol, a major component of wool wax, was not detected. The acyclic triterpene squalene was identified in the hydrocarbon fraction. Normal hydrocarbons which were present are now believed to have originated from an external source (51). It is interesting in this connection to note that the hydrocarbons present in wool wax were found to be natural products of the skin secretion (5). MacKenna et al. (50) rechromatographed the aliphatic alcohol fraction and obtained by crystallisation an impure preparation thought to be eicosanol (?); the acetate of this preparation melted 9 degrees higher than pure eicosanyl acetate. In view of the complexity of the alcohol mixture it was not to be expected that pure components could be isolated by the methods which were used. The same authors isolated some fractions believed to be oxidation products of squalene. A major purpose of their work and a concurrent spectrophotometric investigation by Festenstein and Morton (52) was to examine sebum for the presence of vitamins. No vitamin A, β -carotene, vitamin K, or provitamins D₂ and D₃ were detected. Traces of vitamin E were probably present.

A study of the aliphatic alcohols of sebum by high temperature mass spectrometry has very recently been reported by Brown, Young and Nicolaides (53). The spectra of the consecutive series of saturated alcohols from C₁₆ to C₂₇ were identified and also those of the mono-unsaturated alcohols from C₁₈ to C₂₇. Alcohols having an even number of carbon atoms were present in significantly greater quantity than the adjacent odd homologues. The mass spectrometric method did not distinguish sharply between pure specimens of normal, iso- and anteiso- alcohols, and the authors therefore could give no final decision as to whether iso- and anteiso- alcohols were present in the sebum. A valuable aspect of this method is that a full analysis can be carried out on as little as 30 milligrams of material.

It is likely that various identified constituents of sebum will play a role in connection with studies of skin cancer.

Bernheim et al. (54) have recently shown that the formation of autoxidation products on the skin of mice in vivo (detected by the thiobarbituric acid reagent) is retarded in the presence of certain carcinogenic polycyclic hydrocarbons which are painted on the skin. Further, it is known that carcinogenic hydrocarbons inhibit autoxidation of unsaturated acids in vitro (55); autoxidation of the highly unsaturated hydrocarbon squalene is also inhibited by certain polycyclic hydrocarbons (56). The recently accumulated knowledge of a number of unsaturated compounds which are present in human sebum (acids, aliphatic alcohols, squalene) may therefore lead to renewed studies concerning the interaction between these compounds and carcinogens.

A NEW CONTRIBUTION TO KNOWLEDGE OF SEBUM.

(a) Preliminary investigation.

For a more detailed study of sebum it was necessary to have a large quantity of starting material. It was therefore decided to resort to scalp hair as the source of sebum for the present work, in spite of the possibility that such material could be contaminated with extraneous matter such as commercial hair dressings. Hair dressings consist mostly of neutral glycerides and mineral oils (cf. 45) and such materials do not yield a high percentage of long chain alcohols on saponification. Contamination of the aliphatic alcohols of sebum in the present work is therefore not likely to be appreciable in amount.

Human hair for the present investigation was obtained from African (Bantu) males through the Johannesburg prison authorities. Exhaustive extraction with hot commercial "isooheptane" of different samples of dried hair yielded surface skin "fat" in amounts from 5 to 9 per cent of the hair; (surface skin "fat" will also be referred to as sebum). The following data were determined on a

In similar chromatograms of wool wax unsaponifiables the more strongly adsorbed material had contained a high concentration of 1:2-diols. The appropriate chromatographic fractions of sebum unsaponifiable matter were analysed for glycols, using the volumetric method of periodic acid oxidation by Karnovsky and Rapson (57). A quantity of glycols corresponding to 0.35 per cent of the unsaponifiable matter was found.

The composition of sebum according to these preliminary experiments was in agreement with earlier reports. Hydroxy-acids and dihydric alcohols, however, had not before been reported as constituents of sebum. Because only small quantities of these substances apparently were present, their study was considered of little importance at this stage. It was decided first to investigate the aliphatic alcohol fraction.

(b) The aliphatic alcohols of sebum.

As described in the following, twelve aliphatic alcohols (90) were isolated from sebum and identified as belonging to three homologous series, viz : -

Six normal saturated alcohols,
even numbered :

C_{14} to C_{24}

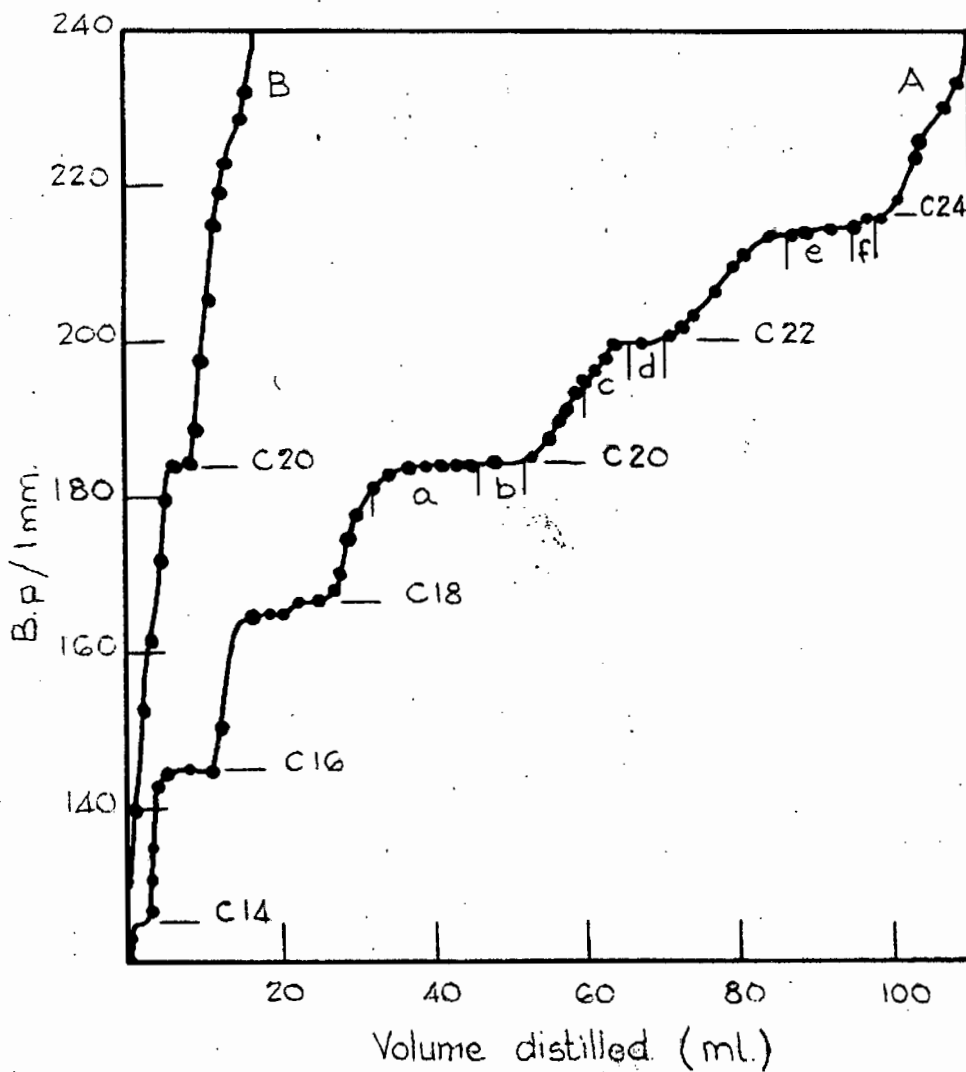
Three saturated iso-alcohols : C_{20} , C_{22} , C_{24} .

Three normal mono-unsaturated alcohols with
double bonds in positions as indicated in
brackets : $C_{20}(10,11)$, $C_{22}(12,13)$, $C_{24}(14,15)$

It is not possible to state with certainty the relative proportion of each alcohol present in the sebum, since intermediate fractions and residues obtained during lengthy fractionations contain unknown amounts of the various compounds; however, an indication of the minimum percentages present is given in the last column of Table I (p. 24).

The normal saturated alcohols isolated are common constituents

Fig. 1



Distillation curves for :

A - acetates of the urea complex forming material of sebum unsaponifiable matter (hydrocarbons excluded);

B - acetates of the residual unsaponifiable matter.

As suggested by the boiling temperatures, the distillation flats contained the acetates of C_{14} , C_{16} , C_{18} , C_{20} , C_{22} and C_{24} alcohols respectively.

The three lower boiling flats each contained the acetate of a normal saturated alcohol; the acetates were purified by crystallisation and the pure alcohols obtained by saponification and crystallisation. Other components were not found in these distillation flats. The three higher boiling flats contained mixtures of acetylated normal, iso- and unsaturated alcohols. Because of a slight difference in boiling point between the three components in these mixtures the acetates of the normal saturated alcohols were enriched in the highest boiling region of the flats and were in two cases (C_{20} and C_{24} alcohols) obtained by crystallisation of material from these regions. The mixtures of acetates were otherwise resolved by fractional crystallisation of urea complexes (cf. 15). The acetates of the normal saturated alcohols had the greatest tendency to form urea complexes; next came the acetates of the saturated iso-alcohols and last the acetates of the normal unsaturated alcohols. The acetates so obtained were saponified and the recovered alcohols crystallised to purity.

One of the unsaturated alcohols (C_{20}) was present in appreciable amount in the residual unsaponifiable matter which did not form a urea complex (Fraction B) and was isolated from this fraction by distillation of the acetates; see curve B, Fig. 1. Iodine values of the distillation fractions indicated that there were unsaturated alcohols present of chain lengths lower than C_{20} and higher than C_{24} , but the quantities were too small for their isolation.

The twelve alcohols which had been isolated were characterised and identified as described in the following. Properties of the alcohols and of four acetates are given in Tables I and II respectively.

Table I. Properties of the isolated alcohols.

Alcohol	M.p. °	C %	H %	X-Ray long crystal spa- cing (Å)	Minimum percentage present _f
n-Tetra- decanol	37.7 - 38.0 38.26 ^a	78.2 (78.4) ^b	14.0 (14.1)	39.6 39.7 ^c	0.2
n-Hexa- decanol	49.3 - 49.4 ^d 49.62 ^a	79.1 (79.3)	14.1 (14.1)	36.8 44.0 37.4 ^c 44.9 ^c	1.4
n-Octa- decanol.	58.0 ^d 57.98 ^a	79.9 (79.9)	14.15 (14.2)	41.2 41.3 ^c 50.2 ^c	2.5
n-Ei- cosanol	65.05 65.5 ^a	80.4 (80.5)	14.2 (14.2)	45.4	3.0
n-Do- cosanol	70.3 - 70.4 70.6 ^a	81.0 (81.0)	14.1 (14.2)	49.8 49.95 ^c	1.3
n-Tetra- cosanol	75.5 - 75.7 74.8 ^a	81.3 (81.3)	14.2 (14.2)	53.2 54.0 ^c	2.0
18-Methyl- nonadecanol	49.2 - 49.4 ^d 50.3 ^e	80.3 (80.5)	14.2 (14.2)		0.5
20-Methyl- heneicosanol	55.4 - 55.9 57.1 ^e	80.9 (80.9)	14.3 (14.2)		0.4
22-Methyl- tricosanol	62.4 - 62.7 62.4 ^e	81.15 (81.3)	14.3 (14.2)		0.5
n-Eicos- 10-enol	23.5 - 24.4	81.0 (81.0)	13.7 (13.6)		0.6
n-Docos- 12-enol		81.4 (81.4)	13.8 (13.7)		0.4
n-Tetracos- 14-enol	43.3 - 43.5	81.6 (81.7)	13.8 (13.7)		0.5

^a Ralston (61).

^b Calculated values for C and H are in brackets.

^c Malkin (62).

^d The m.p. was unchanged by admixture with an authentic sample.

^e Murray and Schoenfeld (14).

^f g./100 g. non-saponifiables.

Table II. Properties of the isolated acetates.

Acetate of	M.p. °	Acetyl %	C %	H %
n-Tetradecanol	13.4 - 14.0	17.0	74.6	12.6
	14.0 ^a	(16.8) ^b	(74.9) ^b	(12.6)
n-Hexadecanol	24.3 - 24.5	15.3	76.1	12.7
	24.2 ^a	(15.1)	(76.0)	(12.8)
n-Octadecanol	32.7 - 32.9	13.9	77.05	13.0
	32.8 ^a	(13.75)	(76.9)	(12.9)
n-Eicosanol	39.7 - 39.8	13.0	77.5	13.0
	40 ± 0.5 ^a	(12.6)	(77.6)	(13.0)

^a Phillips and Mumford (63).

^b Calculated values for acetyl and C and H are in brackets.

Six saturated normal alcohols. These alcohols were identified by their melting points, in two cases mixed melting points with authentic specimens, elementary compositions, X-ray long crystal spacings and in four cases by acetyl values and melting points and elementary compositions of the acetates.

Three saturated iso-alcohols. These were identified by their melting points, elementary compositions and infra-red absorption spectra. The C₂₀ iso-alcohol had an infra-red absorption spectrum which was identical with that of synthetic 18-methylnonadecanol. The terminal iso-propyl structure of the three isolated iso-alcohols was confirmed by the characteristic splitting of the infra-red absorption band in the 725 μ region (64). There was sufficient material for X-ray long crystal spacing measurement of only one of the iso-alcohols (C₂₄). This alcohol gave no clear X-ray pattern and an impurity such as n-tetracosanol might therefore have been present in the sample.

Three unsaturated normal alcohols. These alcohols had one double bond and an unbranched chain as shown by their hydrogenation

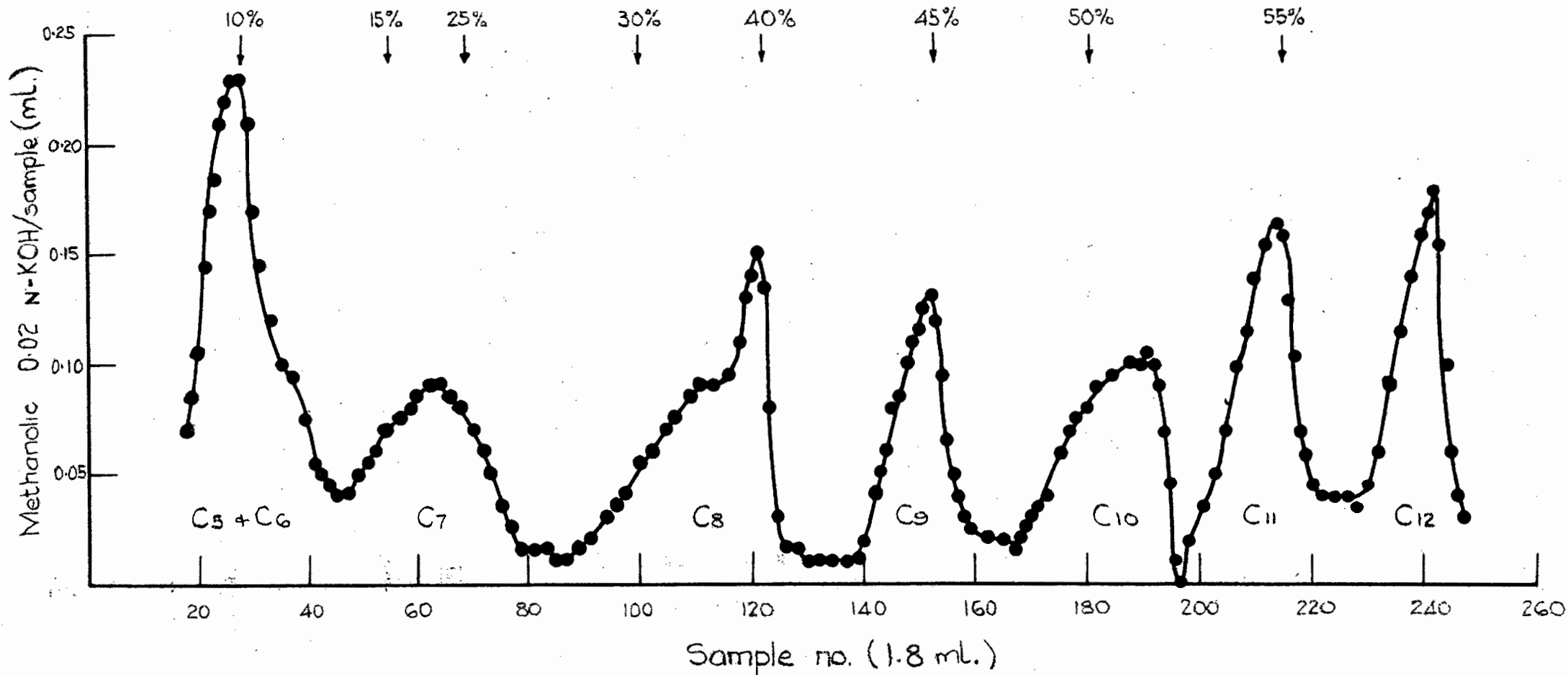
to the corresponding saturated normal alcohols. The double bond positions were determined by oxidative cleavage of the alcohols into two acid fragments, an ω -hydroxy-acid and an unhydroxylated acid, followed by the identification of these acids.

The procedure adopted for oxidative cleavage was chosen so as to minimise the danger of secondary oxidation products being formed. The oxidations were carried out in two steps : the unsaturated alcohols were first oxidised with performic acid in acetic anhydride according to Fietelson's method (65) for oxidation of oleyl alcohol. Trihydroxyalkanes were thereby obtained. The trihydroxyalkanes were next oxidised with lead tetra-acetate and a stream of air according to Mendel and Coops (66, cf. 18) whereby mixtures of ω -hydroxy-acids and unhydroxylated acids were obtained. The acid mixtures were converted to methyl esters and chromatographed on acid-washed alumina, whereby the ω -hydroxy-esters and the unhydroxylated esters were separated. The acids were recovered from the esters by saponification.

The possibility was considered that the unsaturated alcohols were mixtures of double bond position isomers (cf. double bond position isomers in the free fatty acids of sebum (45)) in which case the acid fission products would be homologous mixtures. The technique of reversed-phase partition chromatography of fatty acids ($C_{12} - C_{18}$ acids (67), $C_{20} - C_{24}$ acids (68)) was therefore extended to effect the resolution of six normal fatty acids ranging in chain length from six to eleven carbon atoms. The efficiency of separation is illustrated by the elution curve (Fig. 2) for a chromatogram of a synthetic mixture of n-pentanoic, n-hexanoic, n-heptanoic, n-octanoic, n-nonanoic, n-decanoic, n-undecanoic and n-dodecanoic acids.

The unhydroxylated acid degradation products were analysed by this method. The C_{20} , C_{22} and C_{24} unsaturated alcohols all yielded n-decanoic acid with no traces of its homologues. In the case of the degradation product from the C_{24} alcohol, a small fraction of unidentified material appeared in the eluate in front of the n-decanoic acid. This unidentified material was neither

Fig. 2



Resolution of a mixture of the normal acids C₅ to C₁₂ inclusive.

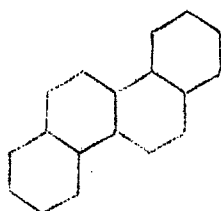
Column 75 x 0.8 cm. Hold-up, 23 ml.
Changes of solvent (% aqueous acetone)
are indicated by the arrows.

a homologue of n-decanoic acid nor was it 14-hydroxytetradecanoic acid as was evidenced by its position in the elution curve and by the melting point of the recovered material. Although this material was not identified, its presence clearly does not influence the argument about the structure of the tetracosenol component. The identification of n-decanoic acid as a degradation product of the C_{20} , C_{22} and C_{24} alcohols proved their structures to be eicos-10-enol, docos-12-enol and tetracos-14-enol respectively.

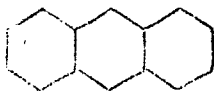
The ω -hydroxy-acid moieties (10-hydroxydecanoic, 12-hydroxydodecanoic and 14-hydroxytetradecanoic acids) proved difficult to purify by crystallisation. It was considered sufficient to identify one of them by oxidation to the dibasic acid : 10-hydroxydecanoic acid was oxidised with potassium permanganate and sebacic acid was recovered from the oxidation product. The recovered acid was not obtained in the highest degree of purity but its identity was confirmed by the running of paper chromatograms (69, 70) in which the unknown acid was compared with sebacic, azelaic and suberic acids.

(c) Polycyclic hydrocarbons in sebum.

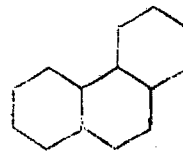
By a chance observation a certain fraction of sebum unsaponifiable matter was found to contain chrysene (I); in following up this work anthracene (II) was also isolated and the presence of phenanthrene (III), pyrene (IV) and fluoranthene (V) was indicated. (91).



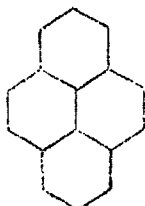
(I)



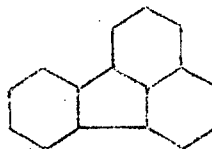
(II)



(III)



(IV)



(V)

From the unsaponifiable matter most of the non-aromatic hydrocarbons, aliphatic alcohols and cholesterol had been removed. The residual material which is earlier referred to as Fraction B had been fractionally distilled and divided into smaller fractions of increasing boiling points (see Fig. 1, p. 22). Some of these fractions deposited small amounts of crystals which fluoresced strongly in ultra-violet light.

The crystalline substance contained in a high boiling fraction (b.p. 198-205°/1 mm.) was isolated and identified as chrysene by means of its ultra-violet absorption spectrum, melting point, mixed melting point with authentic chrysene, melting point of its trinitrobenzene derivative and mixed melting point of the latter with authentic chrysene trinitrobenzene derivative. The ultra-violet spectrum recorded for the isolated compound is given on Fig. 3 (p. 32) together with that of pure chrysene (71).

As shown in Table III certain physical data of chrysene are very similar to those of 3-methylchrysene. The identity of the isolated compound was therefore not fully established by its melting point and ultra-violet spectrum alone. The melting point of the trinitrobenzene derivative definitely excluded 3-methylchrysene.

Table III. Comparative data for chrysene (72), 3-methylchrysene (72) and the hydrocarbon isolated from sebum.

	Hydrocarbon ex sebum		Chrysene		3-Methylchrysene	
M.p. (°C)	253.5		256		254-255	
M.p. of trinitrobenzene derivative	186-187		191		174-176	
Ultra-violet absorption maxima	$m\mu^a$	log ϵ	$m\mu^b$	log ϵ	$m\mu^b$	log ϵ
	258	4.85	258	4.96	260	4.94
	267	5.1	267	5.20	269	5.13
	282	4.04	282	4.09	285	4.00
	294	4.02	293	4.09	297	4.03
	306	4.04	305	4.13	309	4.13
	319	4.04	319	4.13	323	4.15
	344	2.80	343	2.81	345	2.91
	352	2.60	351	2.57	353	2.63
	361	2.71	360	2.81	362	2.91

a in 96% ethanol.

b in abs. ethanol.

The crystalline precipitate of the lowest boiling distillation fraction (b.p. 125-140°/1 mm.) was similarly identified as anthracene by its ultra-violet spectrum, as shown on Fig. 4. The identification was confirmed by the melting point and mixed melting point of the trinitrobenzene derivative.

The isolated preparations of chrysene and anthracene contained trace impurities as was indicated by their spectra and by a green colour of the isolated chrysene and the low melting point of anthracene. It was calculated that the amount of the two hydrocarbons was of the order of 0.01 per cent of the unsaponifiable matter.

The experimental route which led to the isolation of chrysene and anthracene was long and complicated, and a more direct chromatographic method for the detection of polycyclic hydrocarbons in sebum was therefore developed. A sample of the total unsaponifiable matter of sebum was analysed by this method. The presence in separate eluates of phenanthrene, pyrene and fluoranthene was indicated by their ultra-violet absorption spectra. The chromatographic fraction which contained phenanthrene was further purified by reversed-phase partition chromatography on a column of non-wetting kieselguhr. The spectra of the isolated phenanthrene, pyrene and fluoranthene are given on Fig. 5-7 together with those of the corresponding pure hydrocarbons (71). The chromatographic fractions were in most cases too small to be weighed and their recorded absorption intensities are only of qualitative value. Other tests for identification purposes could not be carried out with the minute quantities available.

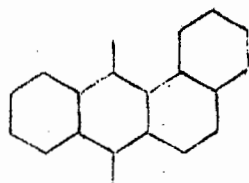
It was evident from ultra-violet spectroscopic data that other polycyclic hydrocarbons were also present.

A small sample of natural hair "fat" from people of European stock living in Pretoria was similarly analysed by the chromatographic method. Ultra-violet spectra of some of the eluates indicated that these contained mixtures of polycyclic hydrocarbons. A fractionation of these mixtures into single components was not attempted.

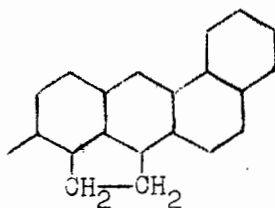
It was thought unlikely that polycyclic hydrocarbons of such wide structural variety were metabolic products secreted through the skin, and possible external sources were considered. While the above work was in progress a paper by Cooper and Lindsey (73) appeared in which the identification of several polycyclic hydrocarbons in atmospheric soot is described. Their discovery offers a plausible explanation for the presence of such hydrocarbons in human sebum: the hair probably acts as an air filter and atmospheric soot is retained by the greasy film on the hairs.

Although this explanation is readily acceptable, it does not exclude the possibility that one or more of the identified hydrocarbons are secreted through the skin. In many studies that have been made of human and animal skins in connection with cancer (74) it has never been observed that fluorescent polycyclic hydrocarbons are natural products of the skin.

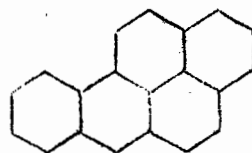
The presence of polycyclic hydrocarbons in surface skin "fat" is of interest in view of the known carcinogenic activity of many hydrocarbons which belong to this class. The most active carcinogens (75) are 9:10-dimethyl-1:2-benzanthracene (VI), 20-methylcholanthrene (VII) and 3:4-benzpyrene (VIII). The latter compound was identified in atmospheric soot by Cooper and Lindsey (73) and can therefore be expected to occur also in human sebum. The five hydrocarbons which have been found so far in sebum are not, or only very slightly, carcinogenic.



(VI)

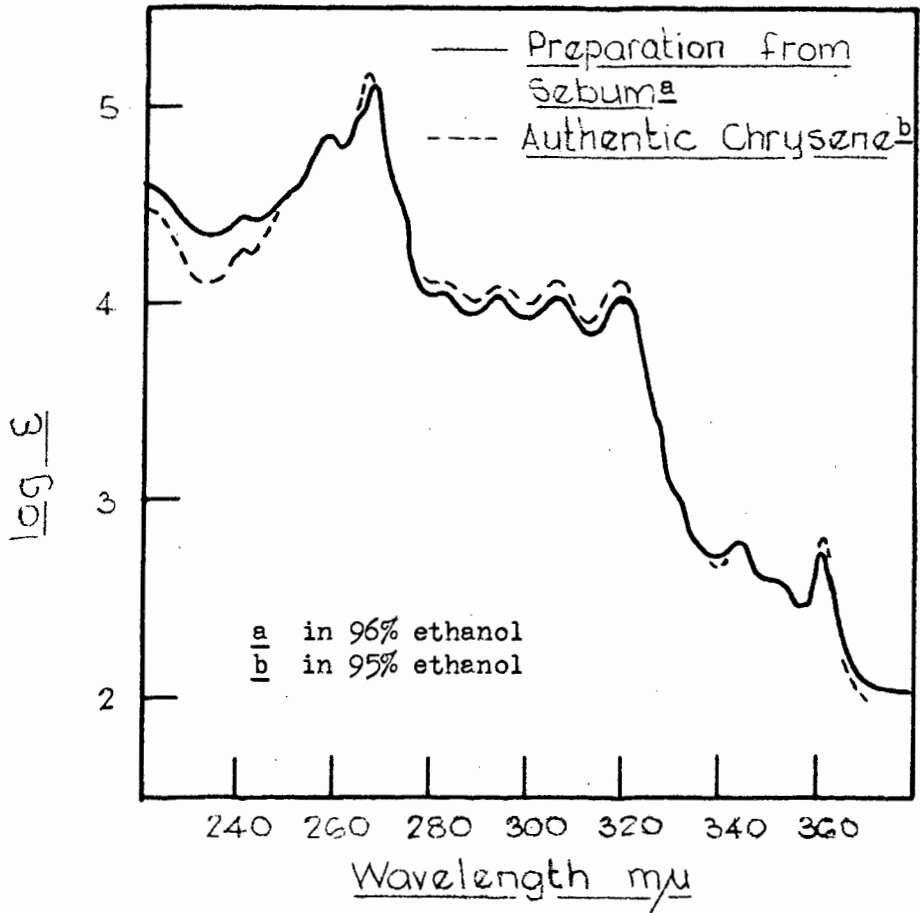


(VII)



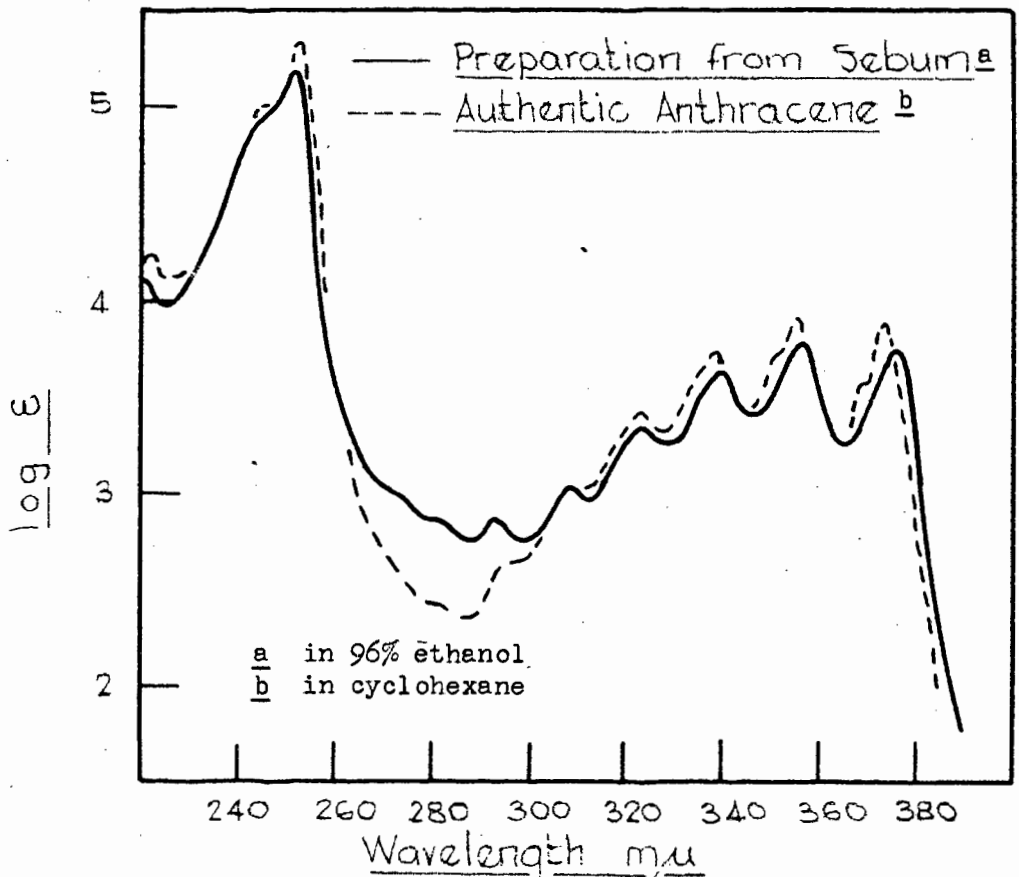
(VIII)

Fig. 3



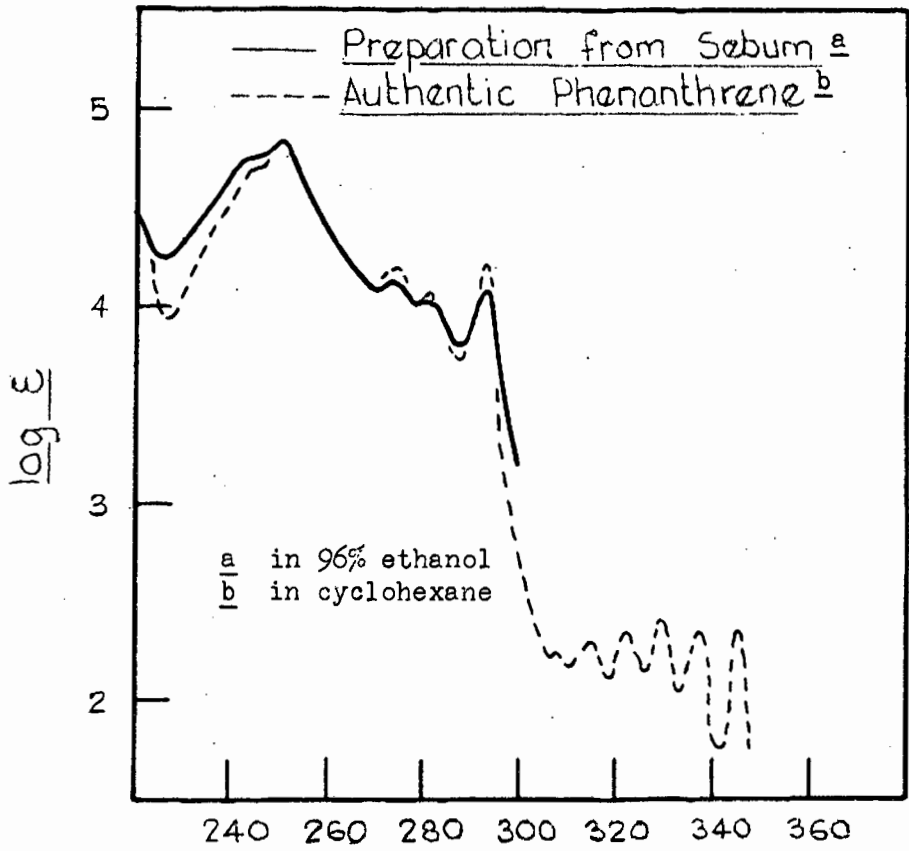
Ultra-violet spectrum of Chrysenes

Fig. 4



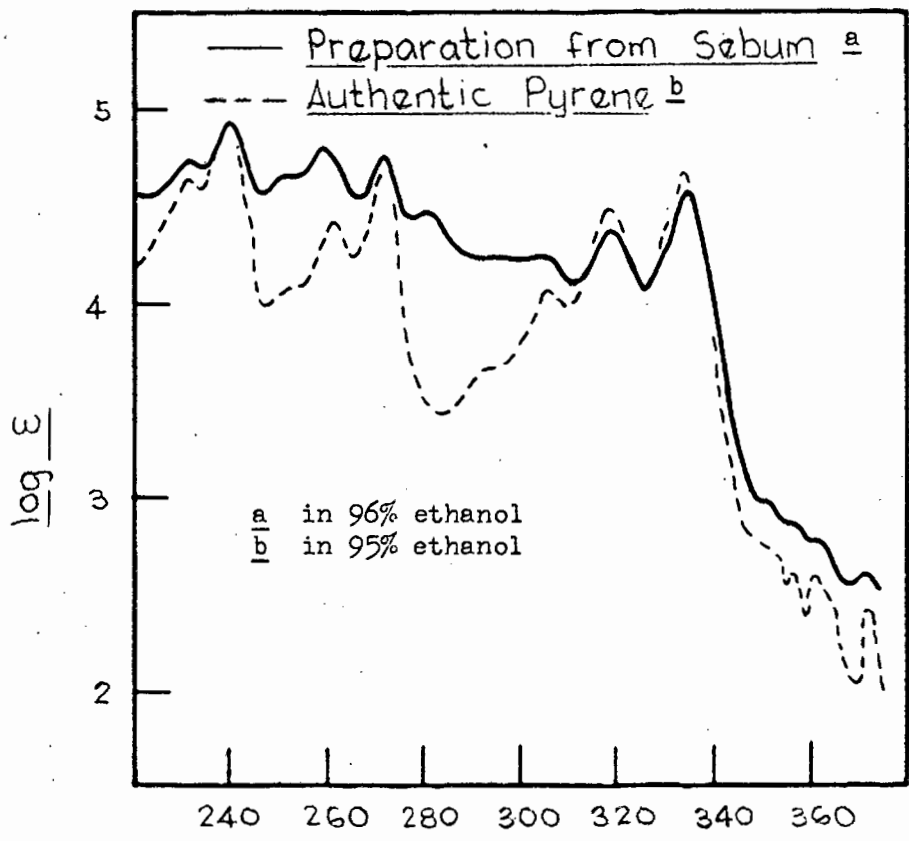
Ultra-violet spectrum of Anthracenes

Fig. 5



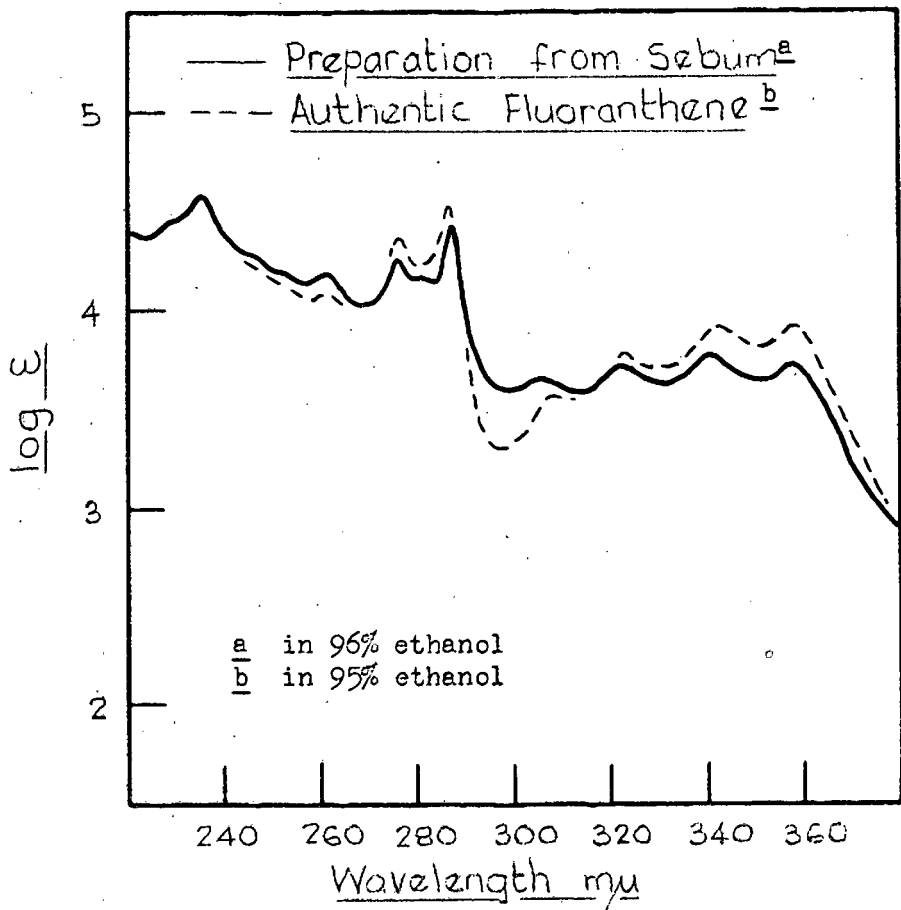
Ultra-violet spectrum of Phenanthrene

Fig. 6



Ultra-violet spectrum of Pyrene

Fig. 7



Ultra-violet spectrum of Fluoranthene

CHAPTER III

SECRETIONS OTHER THAN SEBUM AND WOOL WAX

Such work as has been carried out on materials biologically related to human sebum and wool wax is briefly summarised in this chapter. Most of these products have not yet been investigated in any great detail.

Wheatley (42) has made a comparative study of the following materials of human origin : CERUMEN (ear wax), VERNIX CASEOSA (substance covering the skin of the fetus), OVARIAN DERMOID CYSTS and EPIDERMAL or SEBACEOUS CYSTS. The lipids from these sources are similar to human sebum in that they are waxes containing hydrocarbons and esters of fatty acids, cholesterol and aliphatic alcohols. Phosphatides are also present but these are absent or only occur in traces in sebum (76). Free fatty acids are present in all but the lipids of ovarian dermoid cysts. The aliphatic alcohols of dermoid cyst lipids probably have normal and branched structures, as less than half of the total alcohol fraction formed insoluble urea complexes. Both Wheatley and Cmelik (41) conclude from the chemical composition of sebaceous cyst lipids that these are not of sebaceous origin as is generally accepted from histological observations.

Weitzel (77) has investigated the lipids of the "PREEN" GLANDS from duck, goose and domestic fowl. The preen glands are of sebaceous type and are situated in pairs dorsally at the last vertebra. These are the only skin glands of birds and provide a water-repellent grease with which the bird preens its feathers.

The duck and the goose were found to have similar preen gland secretions. The unseaponifiable matter of both consisted almost exclusively of octadecanol. Most of the acids were branched and some were unsaturated. The major acid components from the duck glands were 4-methylhexanoic acid and a laevorotatory octadecanoic acid. The acids of the goose glands were mainly oleic acid and two laevorotatory branched chain acids having thirteen and fourteen carbon atoms.

The main acid component of the domestic fowl gland was oleic acid. Branched chain acids were not detected. The unsaponifiable matter contained normal alcohols of molecular weight higher than octadecanol. The latter alcohol which is a major constituent of the secretions from duck and goose glands was not detected in the preen glands of the fowl. None of the three kinds of preen glands investigated contained cholesterol or lanosterol; all contained small amounts of glycerol and common fatty acids such as oleic, palmitic and stearic acids.

Eckstein (78) has made a comparative study of the cholesterol contents of surface skin "fats" from a number of animals. Lederer (79) has similarly investigated various skin secretions for their contents of lanosterol. Whilst cholesterol was found in all secretions investigated, lanosterol was present in the secretions of the sheep, goat, llama and camel and absent in those of man, ox, rabbit and hare. Nijkamp (80) has estimated quantitatively by paper chromatography the individual volatile fatty acids in hair "fats" of man and some animals. The volatile acids in dog's hair "fat" have been studied in more detail by the same author (81) and found to consist mainly of iso- and anteiso-pentanoic acids.

A group of animals possess so-called "SCENT" GLANDS, the secretions of which have characteristic and very intense odours and attract the opposite sex of the animal or in some cases repulse enemies. The glands are considered as differentiated sebaceous glands and are situated in the region of the sexual organs. Extensive research has been carried out on some of these glands, primarily because of their contents of valuable perfume ingredients. Lederer has reviewed this field (82). The cyclic ketones muscone and civetone are obtained from the glands of the musk deer and civet cat respectively. "Scent" glands of the American musk rat, the South American alligator, the skunk and the beaver have also been investigated. Castoreum, from the beaver, contains a variety of compounds such as sterols, aromatic alcohols, phenols, aldehydes and an amine.

CHAPTER IV

EXPERIMENTAL

Melting points are corrected.

Ethanol is 96% unless otherwise stated.

Activated alumina for chromatography was prepared by heating alumina (Peter Spence, type H) at 400° for 24 hours with occasional stirring.

The long X-ray spacings were measured on a Philips high-angle Geiger counter spectrometer previously calibrated with a silicon standard. Filtered Cu-K_α radiation was used ($\lambda = 1.541 \text{ \AA}$). The specimens were prepared by melting them between two microscope slides and allowing them to cool under slight pressure.

Ultra-violet absorption spectra were recorded with a Bechman Quartz Spectrophotometer Model DU.

Infra-red absorption spectra were recorded with a Perkin Elmer Spectrophotometer Model 12C; rock salt prism; path length 0.5 mm. Aliphatic alcohols (1.5% solution in CCl₄) were examined in the 7.25 μ region for the presence of iso- and normal structures (64). Unsaturated alcohols (1.5% solution in CS₂) were examined in the 10.35 μ region for trans-configurations and in the 10.1 μ region for terminal double bonds (83).

EXPERIMENTAL RELEVANT TO WOOL WAX.

Extraction of wool wax.

Merino wool (in batches of about 2 kg.) obtained from the Grootfontein College of Agriculture and Experiment Station, Middelburg, Cape, was dried at 60° and 20 mm. pressure for 16 hours (33.61 kg. dried wool) and subsequently extracted continuously for 8 hours with hot commercial "isohexane" in a stainless steel percolator. The extracts were left to stand until the suspended solids settled. The solutions were siphoned off, the solids washed with "isohexane" and the combined solutions evaporated to give crude wool wax (4.975 kg.).

Saponification and separation of acids and unsaponifiable matter.

To a mixture of wool wax (in batches of 500 g.) and hexane (500 ml.) at 40° was added with stirring a solution of KOH (85 g.) in ethanol (1 l.). The mixture was stirred at 40° for 24 hours. To the saponified mixture was added ethanol (660 ml.) and water (1 l.). The solution was shaken three times in a separating funnel with hexane (3 x 2 l.) at 30°. The combined hexane extracts, after being kept overnight in a tall cylindrical separating funnel, were separated from sediment and shaken with 60% ethanol (2 x 500 ml.). Evaporation of the hexane solution yielded extract I (131 g.). The above alcohol solutions were combined and extracted in a continuous liquid-liquid extractor with hexane for 24 hours. The hexane extract was washed with 60% ethanol (200 ml.) and evaporated to yield extract II (59 g.). The alcoholic wash liquors were added to the next batch of saponified wax. The total wax (7000 g.) saponified yielded 38% of unsaponifiable matter (extracts I, 1825 g.; extracts II, 829 g.).

The combined alcoholic solutions of the soluble potassium soaps were diluted with water, acidified (H_2SO_4) and the fatty acids extracted with ether. After drying (Na_2SO_4), the ethereal extracts

were evaporated to yield the main acid fraction. The yield (45-47 g. per 100 g. of wax) was about 90% of the acids present in the wax because acids of low molecular weight which are very soluble in water and acids with very high molecular weight which form potassium soaps insoluble in 60% ethanol were isolated separately.

Isolation of glycols from the unsaponifiable matter by chromatography.

A typical chromatogram was run as follows : Unsaponifiable matter (500 g.) dissolved in wet ether (8 l., saturated with water at 6°), was passed through a column of activated alumina (5 kg., 15 cm. diameter), and eluted with wet ether (24 l.) followed by ether containing 1% of 75% ethanol (20 l.) The eluate was collected in fractions of 2 litres. Those containing the material most strongly adsorbed were analysed by Karnovsky and Rapson's periodate titration method (57) and those containing considerable quantities of diols were amalgamated.

Commercial wool wax unsaponifiables ("Hartolan", Messrs. Croda Ltd., 2.3 kg.) were chromatographed in this way to yield 6.1% of crude glycols (141 g.). The unsaponifiable matter (extracts I and II, 2.2 kg.) of wax from merino wool similarly gave 8.5% of crude glycols (186 g.).

Preparation of methyl esters of the total acids.

Wool wax acids (400 g.) in methanol (1.6 l.) containing H_2SO_4 (25 ml. of 98%) were refluxed for 5 hours. The reaction product was taken up in ether, washed successively with water, a 2% solution of K_2CO_3 and water, dried (Na_2SO_4), and evaporated to yield methyl esters (382 g.).

Reduction of methyl esters with lithium aluminium hydride.

A solution of methyl esters (100 g.) in dry ether (600 ml.) was introduced dropwise with stirring into dry ether (600 ml.) containing $LiAlH_4$ (12 g.) and refluxed for 2 hours. Excess

LiAlH_4 was destroyed with cold 5% H_2SO_4 . The reaction product (99 g.) was recovered by extraction with ether. Two more batches of similar size were reduced; the total methyl esters (271 g.) yielded a mixture of monohydric and dihydric alcohols (243 g.)

Isolation of glycols from reduced wool wax acids by chromatography.

A solution of reduced methyl esters (179 g.) in wet ether (6 l.) was passed through a column of activated alumina (2.12 kg., 10 x 35 cm.) and eluted successively with ether (10 l.) and ether containing 1% of ethanol (4 l.). Ten fractions of 2 litres were collected, samples of which were evaporated to dryness. Appreciable amounts of glycols were contained in fractions 7, 8 and 9 as indicated by their slight solubility in ether. These fractions were evaporated to give crude diols (35 g.).

Preparation of ethyl hydrogen glutarate.

Glutaric acid was synthesised according to Hesse and Bücking (32): condensation of acrylonitrile (26 g.) and diethylmalonate (80 g.) gave crude β -cyanoethylmalonic ester (45 g.), which on decarboxylation and hydrolysis gave crude glutaric acid (27 g.), crystallised from benzene/ether to m.p. 93-96° (lit., 97.5°). Part of the acid was esterified with abs. ethanol and H_2SO_4 in the usual way; ethyl hydrogen glutarate (5 g.) was prepared according to Organic Synthesis (31) from a mixture of glutaric acid, diethylglutarate, dibutylether and HCl.

Preparation of ethyl hydrogen azelate.

Azelaic acid (500 g.) was reacted with abs. ethanol and H_2SO_4 in the usual way to give crude diethylazelate (628 g.). The crude ester (314 g.) was fractionated at 1 mm. pressure through a column equivalent to about 15 theoretical plates. Fractions of b.p.^a 118-119°/1 mm., n_D^{30} 1.4296-1.4300, were combined to give pure diethylazelate (181 g.).

According to Walker (84) the diester (181 g., 0.742 mole) in ethanol (750 ml.) containing the theoretical amount of KOH (41.5 g., 0.742 mole) was refluxed for 5 hours to yield ethyl hydrogen azelate (67 g., n_D^{30} 1.4401) which was separated by distillation (Found: equiv. 214. Calc. for ethyl hydrogen azelate: equiv. 216). Unchanged diester (63 g.) was recovered by distillation and similarly reacted with KOH to yield a second crop of half ester (21.2 g.) (Found: equiv. 218).

Preparation of ethyl hydrogen sebacate.

Crude sebacic acid was treated as described above for azelaic acid to give diethylsebacate (b.p. $130^\circ/1$ mm., n_D^{30} 1.4316-1.4320).

Diethylsebacate (254 g., 0.99 mole) in ethanol (1300 ml.) containing KOH (56 g., 1 mole) was refluxed for 5 hours. The reaction product (196 g.) was recovered in ether and distilled to yield ethyl hydrogen sebacate (113 g.) (Found: equiv. 237. Calc. for ethyl hydrogen sebacate: equiv. 237).

Synthesis of 10-methylundecanoic acid.

The anodic method as described by Linstead (30) under "Method B1" with dry methanol as solvent was used. 3-Methylbutanoic acid (88 g., 0.86 mole), ethyl hydrogen azelate (88 g., 0.41 mole) and sodium (0.6 g., 0.03 g.-atom) were electrolysed. 10-Methylundecanoic acid (37 g., 45% based on the ethyl hydrogen azelate) was recovered by distillation (b.p. $130^\circ/1$ mm.). After crystallisation from ethanol it had m.p. $41.4-41.5^\circ$ and gave no depression of m.p. in admixture with the specimen (m.p. $41.4-41.5^\circ$) supplied by Dr. Weitkamp. X-ray long crystal spacing of the acid was 23.38 \AA .

Synthesis of 18-methylnonadecanoic acid.

The anodic method was used. 10-Methylundecanoic acid (34 g., 0.17 mole), ethyl hydrogen sebacate (113 g., 0.49 mole) and

sodium (0.3 g., 0.01 g.-atom) were electrolysed to yield 18-methylnonadecanoic acid (19 g., 36% based on the 10-methylundecanoic acid), crystallised from ethanol to m.p. 75.3-75.6°, alone or mixed with the specimen (m.p. 75.3-75.5°) supplied by Dr. Weitkamp (Found: C, 76.6; H, 12.9%; equiv., 312. Calc. for $C_{20}H_{40}O_2$: C, 76.7; H, 12.9%; equiv., 312.5).

The acid had X-ray long crystal spacing 37.52 Å. Velick (85) records long spacing 37.8 Å for Weitkamp's (19) acid from wool wax. The amide, prepared from ammonia and the acid chloride in dioxane solution, was crystallised from acetone to m.p. 105.9-106.1° (Weitkamp (19), 105.1°) (Found: N, 4.5. Calc. for $C_{20}H_{41}ON$: N, 4.5%). X-ray long crystal spacing was 36.70 Å (Velick (85), 36.8 Å).

Synthesis of 21-methyldocosanoic acid.

The anodic method was used. 18-Methylnonadecanoic acid (5.5 g., 0.0175 mole), ethyl hydrogen glutarate (4.4 g., 0.027 mole) and sodium (0.02 g., 0.0008 g.-atom) were electrolysed to yield 21-methyldocosanoic acid (0.75 g., 12% based on the 18-methylnonadecanoic acid). Crystallisation from acetone and ethanol gave plates, m.p. 77.9-78.1°, X-ray long crystal spacing 43.9 Å (Found: C, 78.0; H, 13.2%; equiv., 353. $C_{23}H_{46}O_2$ requires C, 77.9; H, 13.1%; equiv., 355).

Synthesis of 19-methyleicosanoic acid.

18-Methylnonadecanoic acid (4.6 g.) was converted to the ethyl ester (4.97 g.) and reduced with lithium aluminium hydride in the usual way to give 18-methylnonadecanol (4.26 g.), crystallised from acetone and hexane to m.p. 50-50.3° (Found: C, 80.5; H, 14.4. Calc. for $C_{20}H_{42}O$: C, 80.5; H, 14.2%). Murray and Schoenfeld (14) record m.p. 50.3° for 18-methylnonadecanol obtained from wool wax.

18-Methylnonadecanol (4 g.), iodine (2 g.) and red phosphorus (0.16 g.) were heated for 5 hours at 145-150° with

occasional shaking. An ethereal extract of the mixture was washed with 5% alkali and water, dried (CaCl_2) and evaporated to yield 18-methylnonadecyl iodide (5.3 g.), plates (from acetone), m.p. 41-43°. A solution of the iodide (4.8 g.) and potassium cyanide (1.6 g.) in 90% ethanol (80 ml.) was refluxed for 24 hours. Potassium hydroxide (10 g.) in water (10 ml.) was added and the solution refluxed for 100 hours. The mixture, after dilution with water, was extracted at 50° with hexane. The alcoholic layer was acidified (HCl), boiled for 10 min. to drive off hydrocyanic acid and extracted with three portions of hexane at 50°. The latter hexane extracts combined were washed with hot water, dried (Na_2SO_4) and concentrated to 50 ml. 19-Methyleicosanoic acid crystallised as plates (2.9 g.). Recrystallised from acetone, it had m. p. 73.1-73.5°, X-ray long crystal spacing 40.2 Å (Found: C, 77.2; H, 13.2%; equiv. 326. $\text{C}_{21}\text{H}_{42}\text{O}_2$ requires C, 77.2; H, 13.0%; equiv., 327).

Craig counter-current distribution separation of a mono- and dicarboxylic acid mixture.

Pimelic acid (0.15 g.) and 10-methylundecanoic acid (0.15 g.) were introduced into tube No. 0 of a 25-tube steel counter-current apparatus containing a two-phase solvent system made up from hexane (100), acetone (25), ethanol (13) and water (62) (figures in parantheses denote parts by volume). After 24 transfers had been applied the contents of the tubes were evaporated, giving pimelic acid, m.p. 105-106°, in tubes 0-4 inclusive (peak in tube 1) and 10-methylundecanoic acid, m.p. 40-41°, in tubes 20-24 inclusive (peak in tube 23).

EXPERIMENTAL RELEVANT TO HUMAN SEBUM.

Some methods of fractionation.

(a) Reversed-phase partition chromatography of fatty acids.

In order to have a method for analysis of shorter chain fatty acids the chromatographic method of Howard and Martin (67, cf. 68) was extended to effect the resolution of the six normal acids C_6 to C_{11} inclusive. The stationary phase was liquid paraffin supported on non-wetting kieselguhr and the moving phase was aqueous acetone. The described procedure (68) was followed except that it was found unnecessary for the present purpose to protect eluate and developing solvent against atmospheric CO_2 ; also, nitrogen was not purified before entering the titration vessel. The column was maintained at tap water temperature ($18-22^\circ$). For loading of the column, the acid mixtures were usually dissolved in the developing solvent. It was necessary to prepare a mull only when the mixed acids differed widely in chain length (such as $C_5 - C_{12}$).

The efficiency of separation is illustrated by a chromatogram of a synthetic mixture of the normal acids C_5 to C_{12} inclusive (about 7 mg. of each), see Fig. 2 (p.27). The C_5 and C_6 acids (unresolved) were eluted with water, C_7 with 10% aqueous acetone (10% acetone in water), C_8 with 25%, C_9 with 40%, C_{10} with 45%, C_{11} with 50% and C_{12} with 55%. The eluate of this particular chromatogram was collected in 1.8 ml. samples and titrated with 0.02N alkali.

(b) Fractional crystallisation of urea complexes.

This method was used for resolving mixtures which contained the acetates of a saturated normal, a saturated iso- and an unsaturated normal alcohol, all having the same number of carbon atoms (cf. 15).

The material (about 10 g.) was dissolved (2% soln.) in benzene containing 35% of abs. ethanol. Urea (in most cases 2 g.) was added to the solution and dissolved under reflux. After standing overnight at room temperature, the solution was filtered from the precipitated urea complex. The filtrate was again treated with urea (2 g.) to yield a second precipitate of urea complex. This procedure was continued until the precipitate consisted of urea only. The complexes were separately decomposed with water and the acetates recovered.

(c) Chromatography on acid-washed alumina.

Alumina (Peter Spence, Grade H) was washed with hot 2% HNO_3 and then with warm water (distilled) and reactivated at 200° .

The unresolved mixture (about 0.5 g.) of an ω -hydroxy-ester and an unhydroxylated ester dissolved in hexane (5 ml.) was passed through a column (1 x 12 cm.) of acid-washed alumina (10 g.) and eluted successively with hexane (35 ml.), hexane containing 10% of dry ether (40 ml.) and dry ether (80 ml.). The eluate was collected in 10 ml. fractions which were evaporated and weighed. The unhydroxylated ester was contained in the hexane eluate and the ω -hydroxy-ester in the ether eluate.

Extraction of sebum.

Scalp hair (28 kg.) from African (Bantu) males was extracted with commercial "isooheptane" in the manner described for wool wax (p. 38) to yield crude sebum (1450 g.).

Determination of the gross composition of sebum.

A sample of crude sebum (100 g., not representative of the total sample) in hexane (500 ml.) was rapidly extracted with a Normal solution of KOH in 50% ethanol (200 ml.). The alcohol solution was repeatedly washed with hexane, acidified (H_2SO_4) and

the FREE FATTY ACIDS (33.05 g.) were recovered with ether.

The acidified alcohol solution was neutralised with barium carbonate. The precipitated BaSO_4 was removed by filtration; the solution was evaporated to dryness to yield WATER-SOLUBLE MATERIAL (15.3 g.), which contained 93.5% ash by ignition.

The combined hexane solutions were evaporated to dryness. The neutral material thus obtained was dissolved in ethanol (160 ml.) containing KOH (8.5 g.) and the solution was kept at 40° for 24 hours with stirring (saponification was complete under these conditions). Water (110 ml.) was added and the solution extracted repeatedly with hexane. The combined hexane extracts were washed with 50% alcohol and evaporated to yield UNSAPONIFIABLE MATTER (29.5 g.).

The combined alcohol solutions were acidified and the "COMBINED" ACIDS (22.1 g.) were recovered with ether.

Counter-current distribution of the "combined" acids.

"Combined" acids (0.358 g.) were introduced into tube No. 0 of a 25-tube steel counter-current apparatus containing the solvent system hexane/80% ethanol (1:1). After 24 transfers had been applied the contents of the tubes were evaporated and weighed. The bulk of material was contained in tubes 12-24 (peak in tube 17) and about 12% in tubes 1-6 (peak in tube 1). Determinations of active hydrogen with LiAlH_4 (86) in n-butylether, were carried out on the material of tube 17 and the combined material of tubes 0-2 inclusive.

Measured evolution of hydrogen (at N.T.P.) per mg. of material :

tube 0-2 : 0.105 ml. H_2
tube 17 : 0.0814 ml. H_2

Assuming acids of same average equivalent weight to be present in tubes 17 and 0-2, the difference in active hydrogen between the two samples can be taken as a measure of hydroxy-acids in tube 17 :

$$\frac{0.105 - 0.0814}{0.0814} 100 = 29\% \text{ hydroxy-acids.}$$

Examination of the unsaponifiable matter (preliminary).

Chromatography. Unsaponifiable matter (29.3 g.) in hexane (600 ml.) was passed through a column of activated alumina (300 g., 4 x 32 cm.) and eluted successively with hexane (4800 ml.), pentane (1600 ml.) containing ether (800 ml.), wet ether (1800 ml.), ether containing 5% of ethanol (1200 ml.) and ether containing 10% of ethanol (1800 ml.). The eluate was collected in 21 fractions of 600 ml. which were evaporated and weighed :

Fractions	1 - 2	7.26 g.	hydrocarbons
"	3 - 9	0.92 "	
"	10 -16	13.35 "	(aliphatic alcohols)
"	17	5.75 "	(cholesterol)
"	18 -21	2.28 "	(glycols)

The hydrocarbon fraction was separated almost quantitatively. There was no sharp separation between aliphatic alcohols, cholesterol and glycols.

Hydrocarbons. The combined chromatographic fractions 1 and 2 (6.25 g., iodine value 102.6) in a mixture of chloroform (10 ml.) and benzene (10 ml.) containing urea (6 g.) moistened with methanol, was shaken for 2½ hours. The precipitate was filtered off and the filtrate was similarly treated twice with urea (2 x 6 g.). The combined precipitates were decomposed in water and worked up in ether to yield straight or slightly branched hydrocarbons (1.23 g., iodine value 62.5). The hydrocarbon fraction (4.86 g.) which was left in the filtrate was recovered in the same way. The latter fraction was distilled at 0.5 mm. pressure into four fractions :

<u>fraction</u>	<u>b.p.(°C)</u>	<u>wt.(g.)</u>	<u>iodine value</u>
1	92	0.17	62
2	120	0.51	37
3	160	1.76	148
4	160-190	0.59	162
residue		1.6	

Fraction 4, in ether, was hydrochlorinated with dry HCl according to Heilbron (87). The hydrochlorinated product, crystallised from acetone, melted at 112-136° (Found : Cl, 28.6. Calc. for $C_{30}H_{56}Cl_6$: Cl, 33.7%). The three isomeric hexahydrochlorides of squalene melt at 107° 113° and 144° (87).

Cholesterol. The chromatographic fraction 17 was crystallised (ethanol, methanol) to m.p. 148.5-149°, unchanged on admixture with cholesterol (Found: C, 84.1; H, 12.0. Calc. for $C_{27}H_{46}O$: C, 83.9; H, 12.0%).

Glycols. The chromatographic fractions 18 and 21 were analysed for 1:2-diols by the gravimetric periodate method of Karnovsky and Rapson (57):

Found : Fraction 18, 3.9% diols
 Fraction 21, 5.8% diols

Assuming 4% diols in fractions 19 and 20, the total amount of diols in fractions 18-21 constitutes 0.35% of the unsaponifiable matter.

Separation of sebum into free acids, "combined" acids and unsaponifiable matter.

A solution of crude sebum (1335 g.) in hexane (8 l.) was extracted in a separating funnel with 60% aqueous ethanol (4 l.) containing KOH (200 g.). The alcoholic extract, after being continuously extracted with hexane, was acidified (H_2SO_4) and the free acids (458 g.) were obtained by extraction with hexane. Further extraction of the alcoholic solution with ether gave a dark resinous material (24 g.). The combined hexane solutions which contained the neutral material were evaporated to a smaller volume (2 l.). Ethanol (2 l.) containing KOH (170 g.) was added and the mixture stirred for 24 hours at 40°. The acids released from combination (285 g.) were separated from the unsaponifiable matter (533 g.) by extraction of the latter with hexane from a solution of the saponified mixture in 60% ethanol (aqueous). A final continuous extraction

combined and referred to as Fraction A) and residual fractions (160 g. and 55 g. respectively) were obtained:

Isolation of cholesterol via its oxalic acid complex.

The method used was essentially that of Pickard and Seymour (60). The above residual fraction (160 g.) which contained cholesterol was dissolved in anhydrous benzene (800 ml.). Anhydrous oxalic acid (16 g.) was added and the solution refluxed for one hour. The precipitated complex was filtered off the next day and washed with cold benzene. It was decomposed with ethanolic KOH solution and cholesterol (29 g.) was recovered in ether. The residual material (125 g.), which was recovered in the same way, was combined with the cholesterol-free residual fraction (55 g.) described in the previous paragraph. This combined material is referred to as Fraction B.

Acetylation of Fractions A (aliphatic alcohols) and B.

Fraction A (116 g.) was heated with acetic anhydride (350 g.) on a water bath for 4 hr. The excess of solvent was distilled off under vacuum to give the acetates (133 g.). A portion of Fraction B was similarly converted to acetates (30 g.).

Distillation of the acetylated Fractions A and B.

Two separate batches (30 g. and 103 g.) of the acetylated Fraction A were distilled at 1 mm. pressure through a Piro and Glover micro spinning band column having an efficiency of about 15 theoretical plates (cf. 18). The distillation curve (A) for the larger batch is shown in Fig. I (p. 22).

The acetylated portion of Fraction B (30 g.) was distilled through the same column (curve B, Fig. I). Only about half of this material distilled over below 240°/1 mm.

Isolation of six saturated normal alcohols.

By crystallisations (from methanol, ethanol or acetone, as suitable) of the appropriate distillation fractions (flats C₁₄,

C₁₆, C₁₈, and fraction b of flat C₂₀ (Fig. 1, p.22), pure acetates of n-tetradecanol, n-hexadecanol, n-octadecanol and n-eicosanol were obtained. Impure n-tetracosanyl acetate was obtained by crystallisation of fraction f of flat C₂₄. Fractional crystallisation of urea complexes was applied to fraction d of flat C₂₂. The first two complexes obtained were recovered to yield an impure preparation of n-docosanyl acetate. The acetates were hydrolysed with ethanolic KOH and the recovered alcohols crystallised to purity (methanol, ethanol, hexane, ether). Analytical data of the alcohols and of four of the acetates are given in Tables I and II (pp. 24, 25).

Isolation of three saturated iso-alcohols.

Fractions a, c and e (Fig. I, p.22), combined with the corresponding distillation fractions from the smaller batch (30 g.) of acetylated Fraction A, were separately fractionated by crystallisation of urea complexes. Fraction a (14 g.) was treated successively with 4 x 10 g. and 4 x 2 g. of urea. The acetate of 18-methylnonadecanol was recovered from the last (eighth) urea complex: the residue left after urea fractionation contained an unsaturated alcohol, as shown by its high iodine value, but this residue was not used as a source of n-eicos-10-enol since a better source (see below) was available. Fractions c (10 g.) and e (8 g.) were treated with 2 g. lots of urea and divided into 13 (numbered c1 - c13) and 11 (numbered e1 - e11) urea complexes respectively. Complexes c8 and e8 were decomposed to give the acetates of 20-methylheneicosanol and 22-methyltricosanol respectively. The acetates were hydrolysed and the recovered iso-alcohols crystallised to purity. Analytical data are given in Table I (p.24).

Isolation and identification of three unsaturated normal alcohols.

n-Eicos-10-enol. The single flat which was obtained by distillation of Fraction B (Fig. I, p.22) contained the acetate of an unsaturated C₂₀ alcohol.

A sample of the crude acetate was hydrogenated (palladium on BaSO₄ in glacial acetic acid) and took up 1.03 moles of hydrogen (based on eicosenyl acetate). The hydrogenated sample melted at 39 - 41°. The acetate was saponified and the recovered alcohol crystallised from hexane and then methanol to m.p. 64.2-64.6° unchanged on admixture with authentic n-eicosanol (Found: C, 80.65; H, 14.4. Calc. for C₂₀H₄₂O: C, 80.5; H, 14.2%).

The total fraction of eicosenyl acetate (about 2 g. from 30 g. of acetylated Fraction B) was saponified and the recovered alcohol (1.95 g.) crystallised from methanol at -60° to m.p. 23.5-24.4° (analysis, see Table I). Examination of the infra-red spectra revealed that the alcohol had the cis-configuration and that there was no terminal double bond or terminal iso-group.

The alcohol (1.3 g.) was oxidised with performic acid in acetic anhydride according to Fietelson's method (65) for the oxidation of oleyl alcohol. The oxidation product was saponified to give crude trihydroxyeicosane (1.29 g.). Crystallisation from a mixture of hexane and methanol and from mixed ether and methanol gave trihydroxyeicosane of m.p. 86.0-86.3° (Found: C, 72.9; H, 12.9. Calc. for C₂₀H₄₂O₃: C, 72.7; H, 12.8%).

This product combined with a less pure second crop from the mother liquors (1.0 g.) was oxidised in benzene solution with lead tetra-acetate and a stream of air according to Mendel and Coops (66, cf. 18). The recovered acid reaction product (0.28 g.) was esterified with methanol/sulphuric acid and the mixed esters (0.27 g.) were separated by chromatography on acid-washed alumina into unhydroxylated ester (0.089 g.) and ω-hydroxy-ester (0.124 g.).

The unhydroxylated ester was hydrolysed to give the acid (0.080 g.). A portion of this acid (±9mg.) was examined by reversed-phase partition chromatography and a single peak was obtained with no traces of neighbouring homologues. A second chromatogram was run with the unhydroxylated acid (4 mg.) in admixture with authentic n-decanoic acid (4 mg.). The total

material was eluted in a single peak and the unhydroxylated acid was therefore n-decanoic acid.

The ω -hydroxy-ester (0.124 g.) was saponified to give the acid (0.116 g.). The acid (in 40% aqueous acetone) was passed through the chromatographic column in order to free it from any admixed unhydroxylated acid. The recovered acid was crystallised from a hexane/benzene mixture to m.p. 69-69.5° (10-hydroxydecanoic acid 75-76°, Chuit and Hausser (88)). The acid was dissolved in aqueous alkali and oxidised with permanganate (5% aqueous) at 50°. The oxidation product was worked up in ether and crystallised from water and extracted with boiling hexane to give a product of m.p. 127-129° (raised to m.p. 130-132° on admixture with sebacic acid of m.p. 133°) (Found: C, 59.8; H, 8.4. Calc. for $C_{10}H_{18}O_4$: C, 59.4; H, 9.0%). Paper chromatograms of C_8 , C_9 and C_{10} dibasic acids and of the acid oxidation product and of the latter in admixture with authentic sebacic acid, were run according to the method of Long, Quayle and Stedman (69), except that the papers were sprayed with bromophenol blue and citric acid (70). The C_8 dibasic acid gave an Rf value of 0.46; C_9 acid, 0.51; C_{10} acid, 0.61; oxidation product, 0.57; a mixture of the oxidation product and C_{10} acid gave a single spot of Rf value 0.58.

n-Docos-12-enol. As already described, fraction c of distillation flat C_{22} (Fig. I, p.22) was divided into 13 urea complexes. The acetate which was recovered from complex c12 was hydrolysed to give an unsaturated C_{22} alcohol (see Table I, p.24). The alcohol had the cis-configuration as shown by its infra-red absorption spectrum and there was no terminal double bond or terminal iso-propyl group.

A sample of the alcohol was hydrogenated and took up 0.88 moles of hydrogen (based on docosenol). The recovered hydrogenated sample was crystallised from ether to m.p. 70.1-70.2°, unchanged on admixture with authentic n-docosanol (Found: C, 81.0; H, 14.3. Calc. for $C_{22}H_{46}O$: C, 80.9; H, 14.2%).

A preparation of docosenol (containing also some 20-methylheneicosanol) was obtained from the combined urea complexes c11 - c13 and the most pentane-soluble part of the recovered alcohols from complexes c9 and c10. This material (1.55 g.) was oxidised with performic acid and the product saponified to give trihydroxydocosane (1.27 g.). Crystallisation from ethyl acetate, followed by many extractions with boiling hexane to remove monohydric alcohol and one crystallisation from ether, gave fine prisms, m.p. 87.4-88.1° (Found: C, 73.9; H, 12.7. Calc. for $C_{22}H_{46}O_3$: C, 73.7; H, 12.9%).

This product was combined with a second crop obtained from the mother liquors (total 0.66 g.) and oxidised with lead tetra-acetate and air. The recovered acid reaction product (0.52 g.) was converted into the methyl esters (0.43 g.) and the latter were chromatographed on acid-washed alumina to give unhydroxylated ester (0.15 g.) and ω -hydroxy-ester (0.2 g.).

The acid (0.093 g.) which was recovered from the unhydroxylated ester was examined by reversed-phase partition chromatography and found to be n-decanoic acid with no traces of neighbouring homologues. A mixed chromatogram with authentic n-decanoic acid confirmed this. The acid which was recovered from the first chromatogram was converted into its p-bromophenacyl ester which was crystallised from aqueous ethanol to m.p. 65.1-65.4° (p-bromophenacyl n-decanoate m.p. 66°, Hopkin and Williams (89)). Admixture with authentic p-bromophenacyl n-decanoate gave no depression of the melting point, whereas authentic p-bromophenacyl n-nonanoate depressed the melting point considerably.

The ω -hydroxy-ester (0.2 g.) was crystallised from pentane to m.p. 33.1-33.8° (methyl 12-hydroxydodecanoate m.p. 34-34.5°, Chuit and Hausser (88)). Chromatography of the ester on acid-washed alumina followed by crystallisation from pentane failed to raise the melting point of the ester. The ester was saponified and the recovered ω -hydroxy-acid crystallised (benzene, hexane/ethyl acetate) to m.p. 78.1-81.7° (12-hydroxydodecanoic acid m.p. 84-85°, Chuit and Hausser (88)).

n-Tetracos-14-enol. As described before, fraction e of distillation flat C₂₄ (Fig. I, p.22) was divided into eleven urea complexes. The acetate contained in complex e10 was saponified to give an unsaturated C₂₄ alcohol. The alcohol was crystallised (methanol, acetone, benzene) to m.p. 43.3-43.5° (see Table I). The infra-red absorption spectrum proved the alcohol to have the cis-configuration and that no terminal double bond or iso-propyl group was present.

A sample of a less pure preparation of the alcohol (m.p. 41.6-42.0°) was hydrogenated and took up 1.06 moles of hydrogen (based on tetracosenol). The hydrogenated sample was recovered and crystallised from ether to m.p. 75.1-75.3°, unchanged on admixture with authentic n-tetracosanol (Found: C, 81.2; H, 14.4. Calc. for C₂₄H₅₀O: C, 81.3; H, 14.1%).

A preparation (1.6 g.) of tetracosanol (containing some 22-methyltricosanol) was obtained from the combined urea complexes e8 - ell. The material was oxidised with performic acid and the product saponified to give trihydroxytetracosane (1.5 g.), crystallised from hexane, ether at -25°, and methanol to m.p. 89-90.4° (Found: C, 74.5; H, 13.1. Calc. for C₂₄H₅₀O₃: C, 74.55; H, 13.0%).

A less pure preparation of trihydroxytetracosane (0.95 g.) was oxidised with lead tetra-acetate and air and the acid oxidation products (0.56 g.) were converted to the methyl esters (0.5 g.). The esters were chromatographed on acid-washed alumina to give unhydroxylated ester (0.15 g.) and ω-hydroxy-ester (0.2 g.).

The unhydroxylated ester was hydrolysed to the acid (65 mg.), and the latter was examined by chromatography on the reversed-phase column. A small fraction of the total material (10-15%) was eluted with 45% acetone and the rest of the material with 50% acetone. The acid contained in the second peak was recovered and converted into its p-bromophenacyl ester, m.p. 65.3-65.5° (p-bromophenacyl n-decanoate, m.p. 66°) (Found: Br, 21.5. Calc. for C₁₈H₂₅O₃Br: Br, 21.7%), unchanged on admixture with an authentic specimen of p-bromophenacyl

n-decanoate. Confirmation that the unhydroxylated acid fraction was substantially n-decanoic acid was obtained by a chromatogram of this material admixed with authentic n-decanoic acid. The smaller fraction of the unhydroxylated acid material which was eluted in front of n-decanoic acid was recovered and melted at 40°.

The ω -hydroxy-ester (0.2 g.) was crystallised from hexane to m.p. 41.7-43.0°. The ester was saponified and the recovered acid crystallised from hexane to m.p. 75-85.5° (14-hydroxytetradecanoic acid, m.p. 91-91.5°, Chuit and Hausser (88)) (Found: C, 68.85; H, 11.6. Calc. for $C_{14}H_{28}O_3$: C, 68.8; H, 11.55%). The acid in 45% aqueous acetone was passed through the reversed-phase column, but there was no improvement in the melting point of the acid recovered from the eluate.

Identification of chrysene and anthracene from sebum.

The acetylated Fraction B (30 g.) of the unsaponifiable matter had been divided into 13 fractions and a residue by distillation. All fractions and the residue fluoresced in ultra-violet light. Small amounts of crystals deposited in fractions 1-3 and 8-11.

The crystals of fraction 9 (b.p. 198-205°/1 mm.) were filtered off (m.p. 249°) and crystallised from methanol/benzene to green plates of m.p. 253.5°, mixed melting point with authentic chrysene 253.5-255.5°, the 2,4,6-trinitrobenzene derivative (prepared in ethanol) melted at 186-187°, unchanged on admixture with authentic chrysene trinitrobenzene derivative. The ultra-violet absorption spectrum of the isolated chrysene was recorded quantitatively in ethanol (Fig. 3, p. 32).

The crystalline precipitate of fraction 1 above (b.p. 125-140°/1 mm.) was filtered off and washed with cold methanol, m.p. 203°. There was insufficient material for recrystallisation. The ultra-violet absorption spectrum was recorded quantitatively in ethanol (Fig. 4, p. 32). The trinitrobenzene derivative, prepared from ethanol, melted at 162.5-163°; the mixed m.p. with authentic anthracene trinitrobenzene derivative was 165-165.2°.

Table IV. Chromatograms of "synthetic unsaponifiable matter".

Frac- tion No.	Solvent	Silica			Alumina		
		Wt. of frac- tions (mg.)	Recovered chry- sene (mg.)	anthra- cene (mg.)	Wt. of frac- tions (mg.)	Recovered chry- sene (mg.)	anthra- cene (mg.)
1	Hexane	0			22.9		
2	"	96.8	0.01	0.06	124.6		
3	"	150.8	0.57	0.53	131.2		
4	"	173.7	0.39	0.26	7.1		
5	"	61.8	0.04	0.01	0.6		
6	"	39.2	0.01		0.6		
7	Benzene	29.5			0.5		
8	"	132.8			4.5	1.07	0.42
9	"	72.5			51.4	0.04	0.03
10	"	94.5			37.8		
11	"	71.8			28.8		
12	Ether	46.0			55.1		
13	"	23.3			102.4		
14	"	12.3			58.9		
15	"	1.3			39.4		
16	"	0.3			23.0		
17	Ethanol/ ether	0.8			25.7		
18	(1:1)	7.8			268.7		
19	"	2.3			2.9		
20	"	1.9			1.5		
21	"				0.8		

Chrysene and anthracene as recovered from the silica column were contained in about five fractions which contained also about 50% of the total "unsaponifiable matter". The alumina column proved far superior for the isolation of these hydrocarbons which from this column were recovered substantially in one fraction. This fraction contained only 0.5% of the total "unsaponifiable matter".

The incomplete recovery of anthracene from the chromatograms (see Table IV) was apparently caused by evaporation of this substance when the chromatographic eluates were evaporated and dried : anthracene (0.1 mg.) in ethanol (10 ml.) was evaporated on a water-bath until only a few drops of solution were left in the flask. The contents of anthracene in the flask were then 0.04 mg.; i.e. ca. 60% of the anthracene had evaporated. It was found that the solvent could be evaporated at 30° and 20 mm. pressure and the

sample dried for a further half hour under the same conditions without any appreciable loss of anthracene.

Penanthrene, pyrene and fluoranthene from Bantu sebum by the chromatographic method.

Unsaponifiable matter (1 g.) of Bantu sebum was chromatographed on a column of activated alumina as described for "synthetic unsaponifiable matter" in the preceding paragraph. The eluates corresponding to fractions 6, 7 and 8 of Table IV and an analogous fraction from a similar chromatogram of sebum unsaponifiables were combined (total 68 mg.) and rechromatographed on alumina (10 g., 1 x 12 cm.) as follows :

<u>Fraction no.</u>	<u>Solvent</u>	<u>Volume per fraction (ml.)</u>
1	hexane	30
2	hexane/benzene (10:1)	30
3	" "	10
4-5	" (5:1)	10
6-13	" "	5
14-20	" (5:2)	5
21-32	" (1:1)	5
33-36	benzene	10
37	ethanol/ether (1:1)	50

The eluates, which were evaporated to dryness, in most cases weighed only a fraction of a milligram. Fractions 3-37 fluoresced in ultra-violet light. Ultra-violet spectra of the eluates were recorded and these indicated the presence of phenanthrene in fraction 4, pyrene in fractions 5 and 6 and fluoranthene in fractions 8-10.

The combined fractions 3 and 4 were rechromatographed on a reversed-phase column of non-wetting kieselguhr (10 g.) with heptane as stationary phase and 50% aqueous ethanol as moving phase.* Some purification of the phenanthrene was achieved, but the quantity obtained was too small for the recording of its ultra-violet absorption at the longer wavelengths.

The recorded spectra (in ethanol) of the purified phenanthrene and the above fractions 5 (pyrene) and 9 (fluoranthene) are given

* The application of this method for purification of polycyclic hydrocarbons was adopted from Dr. P.R. Enslin of this laboratory.

in Fig. 5-7 (pp. 33 & 34) together with the spectra of the corresponding pure hydrocarbons (71). The maximum quantities of hydrocarbons which were present in the respective chromatographic fractions are calculated from the absorption intensities :

phenanthrene in fraction 4 :	0.03 mg.
pyrene in fraction 5 :	0.6 "
fluoranthene in fraction 9 :	0.3 "

Polycyclic hydrocarbons in sebum from people of European stock.

European hair was collected from a ladies' hairdressing saloon in Pretoria. Care was taken to include hair only from customers who used no hair dressings of any kind. The extraction of sebum, saponification and isolation of unsaponifiable matter were carried out as previously described for Bantu sebum. The unsaponifiable matter (5 g.) was chromatographed on activated alumina. All except the first fractions which were eluted with hexane fluoresced in ultra-violet light. The ultra-violet spectra of some of these fractions indicated the presence of mixtures of polycyclic hydrocarbons.

SUMMARY

(1) The chemistry of wool wax has been reviewed and the present advances to which the author has contributed are included. The latter are investigations into the dihydric alcohols and α -hydroxy-acids and the syntheses of some iso-acids which occur in wool wax.

Five dihydric alcohols have been identified : n-hexadecane-, 16-methylheptadecane-, 18-methylnonadecane-, 20-methylheneicosane--and 22-methyltricosane-1:2-diols.

Five α -hydroxy-acids have been identified : 2-hydroxy-n-dodecanoic, 2-hydroxy-n-tetradecanoic, 2-hydroxy-n-hexadecanoic, 2-hydroxy-n-octadecanoic and 2-hydroxy-16-methyl-heptadecanoic acids.

Five iso-acids have been synthesised : 8-methyl-nonanoic, 10-methyl-undecanoic, 12-methyl-tridecanoic, 16-methylheptadecanoic and 18-methylnonadecanoic acids.

(2) The chemistry of human sebum has been reviewed. The present study concerns the aliphatic alcohols and a group of polycyclic hydrocarbons which occurred in sebum.

Twelve alcohols were identified : n-tetradecanol, n-hexadecanol, n-octadecanol, n-eicosanol, n-docosanol, n-tetracosanol, 18-methylnonadecanol, 20-methylheneicosanol, 22-methyltricosanol, n-eicos-10-enol, n-docos-12-enol and n-tetracos-14-enol. The three unsaturated alcohols have not been described before.

Trace amounts of polycyclic hydrocarbons were found in sebum; these are believed to originate from atmospheric soot. Chrysene and anthracene were identified; the presence of phenanthrene, pyrene and fluoranthene was strongly indicated.

(3) In order to provide a method for the analysis of small amounts of shorter chain fatty acids, the reversed-phase partition chromatographic method of Howard and Martin for the analysis of C_{12} to C_{18} acid mixtures was extended to include the separation of the six normal fatty acids C_6 to C_{11} inclusive.

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ACKNOWLEDGEMENTS

The author wishes to thank his three supervisors; professor W. Pugh and professor F.G. Holliman of the University of Cape Town and Dr. W.S. Rapson of the National Chemical Research Laboratory, Council for Scientific and Industrial Research, who also suggested this problem. Dr. D.A. Sutton and Dr. D.H.S. Horn assisted the author with beneficial discussion.

The work described in this thesis was carried out in the laboratories of the South African Council for Scientific and Industrial Research and the author is appreciative of the facilities accorded to him.

POLYCYCLIC HYDROCARBONS IN HUMAN HAIR WAX

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The recent communication of Cooper and Lindsey¹ who found a wide variety of polycyclic hydrocarbons in atmospheric soot, prompts us to record some recent observations.

A large sample of human hair wax was obtained by extraction of the hair from many Bantu (negro) people living in Johannesburg. The wax was saponified. After removal of the paraffins and most of the fatty alcohols and cholesterol from the non-saponifiables (chromatography, urea complex formation, oxalic acid complex formation), the residue was distilled *in vacuo*. After standing, several fractions deposited crystals showing a strong fluorescence in ultra-violet light. The crystals from a low and a high boiling fraction (b.p. 125—140°/1 mm. and 198—205°/1 mm.) were isolated and had ultra-violet spectra indicating that they were most probably anthracene and chrysene respectively; this evidence alone does not exclude closely related analogues (e.g. 3-methylchrysene).

In separate experiments the non-saponifiables were chromatographed on alumina. The fluorescent fractions (in ultra-violet light) were rechromatographed and the ultra-violet spectra of the resultant sub-fractions were recorded. Indications of the presence of phenanthrene, pyrene and fluoranthene were thereby obtained²; in addition other polycyclic hydrocarbons were shown to be present.

Hair wax from people of European stock living in Pretoria is being similarly investigated. Polycyclic hydrocarbons were found in this material also, although in smaller quantity.

From the variety of polycyclic hydrocarbons found, it seems unlikely that these are all metabolic products. In view of the recent work of Cooper and Lindsey,¹ a more plausible explanation is that the hair acts as an air filter and is, therefore, a good collector of atmospheric soot. Natural or added grease on hair surfaces would tend to retain this soot. We intend, nevertheless, to seek further evidence as to the origin of the polycyclic hydrocarbons.

The author thanks Dr. D. A. Sutton for his interest in the work and the South African Council for Scientific and Industrial Research for permission to publish this communication.

Received January 4, 1954

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CONSTITUTION OF THE HYDROXYACIDS OF WOOL WAX

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Earlier investigations^{1, 2, 3, 4} of the acidic fraction of wool wax have shown the presence of small quantities of hydroxylated fatty acids. Thus Kuwata² obtained impure *n*-2-hydroxyhexadecanoic acid in a yield of 7% of the mixed wool wax acids. Later, the presence of this acid was confirmed by Weitkamp³, who isolated it in 4% yield of the acids distilled. The latter author also isolated *n*-2-hydroxytetradecanoic acid in a 0.2% yield. On the other hand, Bertram⁵ has inferred from analytical data that "the hydroxy acids are not only the most characteristic acids but also constitute the major part of them".

The present study of the acidic fraction of wool wax extracted from merino fleeces has revealed the presence of nearly 30% of hydroxylated fatty acids. The major components of this mixture of hydroxyacids have been characterized in two ways. In one, the mixture of hydroxyacids has been separated almost quantitatively from the unhydroxylated acids and resolved into its several components by means of the Craig solvent distribution method, and in the other, the total mixture of esterified acids has been converted with lithium aluminium hydride to the corresponding alcohols, the dihydric alcohols separated from the monohydric alcohols by chromatography on activated alumina, and finally the mixed diols resolved by fractional distillation of their acetates at reduced pressure in the manner described previously.⁶ In this way, the presence of the laevorotatory α -hydroxyacids shown in Table I has been established.

The mixture of 2-hydroxyoctadecanoic acid isomers has not as yet been resolved into its pure components, but it has been deduced from an infra-red examination that the main isomer present is 16-methyl-2-hydroxyheptadecanoic acid. It is suspected that the other isomer present is *n*-2-hydroxyoctadecanoic acid.

Besides the above acids, a small quantity of much higher molecular weight hydroxylated acids, similar to those described previously,^{4, 7} were also isolated. However, the material proved to be a complex mixture and has not been further investigated.

The authors thank Dr. W. S. Rapson and Dr. D. A. Sutton for their interest in the work and the Council for Scientific and Industrial Research for permission to publish this preliminary note. The investigation constitutes part of a project sponsored by the South African Wool Board.

Received December 20, 1952

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Table I

α -Hydroxyacid	Per centage of mixed acids	M.p. °C.		M.p. °C.		[α] _D ²⁰ CHCl ₃ acid
		Present study	Me Ester	Literature	Me Ester	
1. <i>n</i> -2-Hydroxydodecanoic acid (<i>n</i> - α -hydroxy-lauric)	0.6	78-78.5	24-25	—	—	-2.8°
2. <i>n</i> -2-Hydroxytetradecanoic acid (<i>n</i> - α -hydroxymyristic)	3.8	88.2	34.8	88.5 ³	—	-3.1°
3. <i>n</i> -2-Hydroxyhexadecanoic acid (<i>n</i> - α -hydroxypalmitic)	18.8	93.5	45.5-45.7	86-87 ²	45-62 ²	-3.2°
4. 2-Hydroxyoctadecanoic acid isomers (α -hydroxystearic isomers)	4.6	76.5-77.5	38.5-39.5	93.6 ³	45.6 ³	-3.8°
Total	27.8					

THE ISOLATION OF SOME HOMOLOGOUS HIGHER MOLECULAR WEIGHT GLYCOLS FROM THE UNSAPONIFIABLE FRACTION OF WOOL WAX

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A chromatographic separation of the unsaponifiable material of wool wax has revealed the presence of an interesting new series of organic components. These substances, now characterized as alkyl-1 : 2-diols, have been isolated from that portion of the unsaponifiable material which is relatively strongly adsorbed on activated alumina from an ether solution, by fractional distillation of the acetate of this material through a small spinning band fractionating column. Under similar conditions of operation, this still has been found to have an

that of the natural C_{18} diol. It, however, showed a considerable melting point depression with the corresponding natural diols indicating that the natural compounds are not of the same series, although the X-ray long spacing measurements suggest the two molecules to be of similar length. It seems likely, therefore, that the alkyl chains of these natural diols may have a single methyl branch, as occurs in the iso and anteiso fatty acids present in wool wax³. In this respect, it is interesting to note that the curve of the melting points of the natural glycols plotted against the carbon content passes through a minimum at C_{18} , for melting point curves exhibiting maxima and minima were also encountered in the case of the iso and anteiso fatty acid amide melting point diagrams.⁴

The combined diols were first isolated in about 2% yield from commercial wool wax unsaponifiables,⁵ but have since been obtained in about 4-5% yield from a sample of the unsaponifiables

Table I

Proposed acetate formulae	B.p. 1 mm. ± 0.05 mm.	M.p. ° c.	% Acetyl		% Carbon		% Hydrogen	
			Found	Calc.	Found	Calc.	Found	Calc.
$C_{30}H_{58}O_4$	173-5							
$C_{28}H_{54}O_4$	189-90	16-7	23.1	23.2	71.2	71.3	11.6	11.4
$C_{26}H_{50}O_4$	209-211	26-7	21.9	21.6	72.65	72.3	11.7	11.6
$C_{24}H_{46}O_4$	220-222	33.3-33.8	20.4	20.2	73.2	73.2	11.9	11.8
$C_{22}H_{42}O_4$	232.5	37.5-38.5	19.0	18.9	74.1	73.9	12.0	12.0
1 : 2-diacetoxy- <i>n</i> -octadecane (synthetic)—								
$C_{28}H_{54}O_4$	192	38.5-38.7	23.3	23.2	71.5	71.3	11.6	11.4

Table II

Proposed diol formulae	M.p. ° c.	% Carbon		% Hydrogen		Mol. Weight by HIO_4	X-ray long spacing d [Å]
		Found	Calc.	Found	Calc.		
$C_{16}H_{34}O_2$	83.7-84.4	74.6	74.4	13.2	13.3	261	258
$C_{18}H_{38}O_2$	74.0-75	75.8	75.5	13.4	13.4	288	287
$C_{20}H_{42}O_2$	80.5-81.0	76.6	76.4	13.6	13.5	322	315
$C_{22}H_{46}O_2$	84.0-84.5	77.1	77.1	13.6	13.5	342	343
$C_{24}H_{50}O_2$	85.5-86.0	78.1	77.8	13.6	13.6	360	371
<i>n</i> -octadecane-1 : 2-diol (synthetic)—							
$C_{18}H_{38}O_2$	81.0-81.8	75.6	75.5	13.5	13.4	285	287

efficiency equivalent to about fifteen theoretical plates. The test mixture, *n*-butyl azelate and *n*-butyl phthalate of Williams¹ was used.

For analysis the five main fractions were crystallized to purity from methanol. The pure compounds crystallized from this solvent in needles. No appreciable optical activity was found.

The properties and analyses of the five pure acetates are summarized in Table I.

The respective acetates were saponified to yield the corresponding diols, the properties of which are summarized in Table II.

The molecular weight of each diol was found by oxidation with periodic acid according to the method of M. L. Karnovsky and W. S. Rapson.² Under these conditions, each diol was found to yield nearly one equivalent of formaldehyde, thus identifying them as 1 : 2-diols.

For comparison with the natural diols, *n*-octadecane-1 : 2-diol was synthesized. It was found to have similar properties to

prepared from the wax extracted from a local merino fleece. T. Kuwata and M. Katuno³ have also reported the presence, in wool wax unsaponifiables, of a dihydric alcohol, for which they propose the formula $C_{21}H_{40}(OH)_2$.

The authors thank Mr. F. W. G. Schöning for carrying out the micro-analyses, Mr. L. Schöning for measuring the X-ray long spacings, Drs. W. S. Rapson and D. A. Sutton for their interest in the work and the Council for Scientific and Industrial Research for permission to publish this preliminary note.

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710. Wool Wax. Part IV.* The Constitution of the Aliphatic Diols.

By D. H. S. HORN and F. W. HOUGEN.

Five alkane-1 : 2-diols have been isolated from the unsaponifiable fraction of wool wax by chromatography and distillation at reduced pressure. They are identified, from infra-red studies and by degradation to fatty acids of known constitution, as *n*-hexadecane-, 16-methylheptadecane-, 18-methylnonadecane-, 20-methylheneicosane-, and 22-methyltricosane-1 : 2-diol.

KUWATA and KATUNO (*J. Soc. Chem. Ind. Japan, Suppl.*, 1938, **41**, 227) isolated from the unsaponifiable fraction of wool wax a dihydric alcohol, m. p. 79—80°, for which they proposed the formula $C_{21}H_{40}(OH)_2$. During a chromatographic study of the unsaponifiable fraction of wool wax on activated alumina, we isolated a similar material from the most strongly adsorbed fraction. However, attempts to purify it revealed that it was a complex mixture of closely related compounds, differing principally in molecular weight.

Oxidation with lead tetra-acetate yielded formaldehyde, characterised as its dimerone compound, and a mixture of aliphatic aldehydes which was readily converted, on mild oxidation, into the corresponding saturated fatty acids. These preliminary investigations established the main components as alkane-1 : 2-diols (Horn and Hougen, *Chem. and Ind.*, 1951, 670). Murray and Schönfeld later (*J. Amer. Oil Chem. Soc.*, 1952, **29**, 416) also reported the isolation of some diol fractions.

Initially, the diols were prepared in 2% yield from commercial wool wax unsaponifiables ("Hartolan," Messrs. Croda Ltd.). Subsequently, they were obtained in 4—5% yield from the unsaponifiable material prepared from wool wax extracted with *iso*heptane from merino wool, kindly supplied by the Director of the Grootfontein College of Agriculture and Experiment Station. To avoid the production of artefacts (Daniel, Lederer, and Velluz, *Bull. Soc. Chim. biol.*, 1945, **27**, 218), saponification of the wax was carried out at 40°. The unsaponifiable material was separated, almost quantitatively, by a method similar to that of Barnes, Curtis, and Hatt (*Austral. J. Appl. Sci.*, 1952, **3**, 88).

After the failure of attempts to resolve the diol mixture by fractional crystallisation and chromatography, the acetylated material was fractionally distilled at reduced pressure in a spinning-band microstill. In the initial stages of the work, the acetylated mixture was distilled without a break (Fig. 1). Later, with more material available, it was more profitable to fractionate it roughly at a low reflux ratio into several arbitrary fractions, redistil each fraction more thoroughly, and combine appropriate distillates. In this way, decomposition was reduced to a minimum. Finally, the main components were separately distilled. This lengthy procedure was necessary because chromatography and crystallisation failed to remove completely the long-chain alcohols, the presence of which greatly complicated the separation of, especially, the higher components (see Fig. 1) and made it impossible to calculate accurately the relative proportions of the diols present.

The following pure diols were obtained by saponification and crystallisation of the pure fractions: *n*-hexadecane-, 16-methylheptadecane-, 18-methylnonadecane-, 20-methylheneicosane-, and 22-methyltricosane-1 : 2-diol. No other diols could be detected: if present, they occurred only in very small amounts and were lost in the intermediate fractions or did not survive the lengthy distillation.

The structures of the individual diols were determined by direct oxidation with an excess of lead tetra-acetate in a stream of air (cf. Mendel and Coops, *Rec. Trav. chim.*, 1939, **58**, 1140) to the corresponding fatty acids having one methylene group less than the parent

* Part III, *J.*, 1953, 98.

diols. The acids produced were compared with synthetically prepared acids of known structure.

(\pm)-*n*-Octadecane-1 : 2-diol required for comparison was synthesised from 1-octadecene (Wibaut, *Rec. Trav. chim.*, 1948, **67**, 113; cf. Minoru *et al.*, *J. Chem. Soc. Japan*, 1949, **52**, 50; *Chem. Abs.*, 1951, **45**, 4635) by a modification of the method of Swern, Billen, and Scanlan (*J. Amer. Chem. Soc.*, 1946, **68**, 1505).

The structures of the pure diols were further confirmed by making use of Thompson and Torkington's observation (*Trans. Faraday Soc.*, 1945, **41**, 246; cf. Sobotka and Stynler, *J. Amer. Chem. Soc.*, 1950, **72**, 5139; Freeman, *ibid.*, 1952, **74**, 2526) that a doublet in the 1360—1400-cm.⁻¹ range of the infra-red absorption spectra of alkanes is characteristic of the isopropyl group. Hexadecane-1 : 2-diol showed a single absorption band, identical with that of synthetic (\pm)-octadecane-1 : 2-diol (Fig. 2), at 1380 cm.⁻¹. The other pure wool wax diols showed the characteristic splitting of the 1380 cm.⁻¹ band (Fig. 2), and the intensity of absorption was that expected for a single methyl branch.

The optical rotations of the pure diols could not be obtained with certainty because of their low solubilities. However, in contrast to (\pm)-octadecane-1 : 2-diol, they formed well-defined crystals and their diacetates had a slight dextrorotation. They have been

FIG. 1. Distillation curve

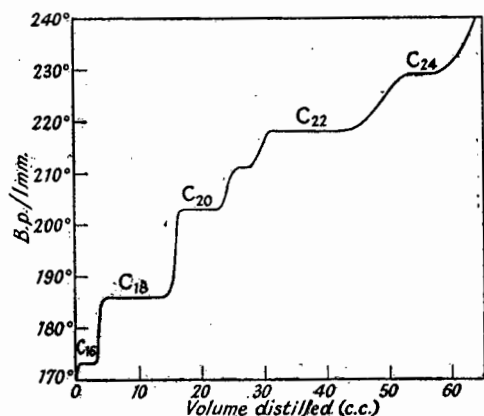
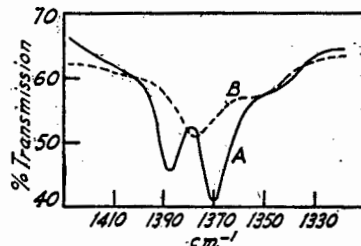


FIG. 2. Infra-red absorption.



A, 16-Methylheptadecane-1 : 2-diol.
B, Octadecane-1 : 2-diol.

correlated configurationally with the laevorotatory hydroxy-acids which occur in the wool-wax acid fraction (Horn, Hougen, and von Rudloff, *Chem. and Ind.*, 1953, 106). Thus on reduction with lithium aluminium hydride ($-$)-*n*-2-hydroxyhexadecanoic acid* and ($-$)-2-hydroxy-16-methylheptadecanoic acid yielded diols, respectively identical with *n*-hexadecane-1 : 2-diol and 16-methylheptadecane-1 : 2-diol obtained from the unsaponifiable material.

EXPERIMENTAL

Crystal long spacings were measured as described previously (Part III, *loc. cit.*). M. p. determinations were made with a heating rate of 3—4 min./degree in a "Hershberg"-type apparatus. Standardised, 8-cm., immersion thermometers were used.

The infra-red absorption spectra were obtained with the Perkin-Elmer spectrophotometer model 12C. An approx. 2% solution of the diol in carbon tetrachloride was run into a 0.5-mm. rock-salt cell warmed with an infra-red lamp. In this way, the diol could be kept in solution long enough after the lamp had been switched off to allow the absorption curve to be obtained.

Extraction of Wool Wax from Merino Fleeces.—Wool (2—3 kg.) was dried for 16 hr. at 60—70°/14—20 mm., and extracted for 7 hr. with isoheptane in a stainless-steel hot extractor. After storage for several days, the cold extract was decanted from the sludge and evaporated to dryness. In this way, 33.61 kg. of dried wool yielded 4.975 kg. (14.8%) of wool wax.

Saponification and Isolation of the Unsaponifiable Material.—To a mixture of wool wax (500 g.) and hexane (500 c.c.), at 40°, was added, with stirring, a solution of potassium hydroxide

* Genera numbering, CO₂H = 1.

20-Methylheicosane-1 : 2-diol. The foregoing diacetate was saponified. The diol crystallised from hexane and methanol as plates, m. p. 84.0—84.4°, crystal long spacing 48.6 Å (Found : C, 77.1; H, 13.6%; *M*, 342. $C_{22}H_{46}O_2$ requires C, 77.1; H, 13.5%; *M*, 343).

Synthesis of 19-methyleicosanoic acid. 18-Methylnonadecanoic acid (Part III, *loc. cit.*), reduced with lithium aluminium hydride in the usual way, yielded 18-methylnonadecanol, plates (from acetone and hexane), m. p. 50—50.3° (Found : C, 80.5; H, 14.4. Calc. for $C_{20}H_{42}O$: C, 80.5; H, 14.2%). Murray and Schoenfeld (*J. Amer. Oil Chem. Soc.*, 1952, 29, 416) record m. p. 50.3° for 18-methylnonadecanol obtained from wool wax.

18-Methylnonadecanol (4 g.), iodine (2 g.), and red phosphorus (0.16 g.) were heated for 5 hr. at 145—150° with occasional shaking. An ethereal extract of the mixture was then washed free from acid, dried ($CaCl_2$), and evaporated, to yield 18-methylnonadecyl iodide (5.3 g.), plates (from acetone), m. p. 41—43°. A solution of this (4.8 g.) and potassium cyanide (1.6 g.) in 90% ethanol (80 c.c.) was refluxed for 24 hr. Potassium hydroxide (10 g.) in water (10 c.c.) was then added and the solution refluxed for 100 hr. The mixture, after dilution with water, was extracted at 50° with hexane. The alcoholic layer was acidified with hydrochloric acid, boiled for 10 min. and then extracted with three portions of hexane at 50°. The combined hexane extracts were washed with hot water, dried (Na_2SO_4), and concentrated to 50 c.c. The 19-methyleicosanoic acid then crystallised as plates (2.9 g.). Recrystallised from acetone, it had m. p. 73—73.3°, crystal long spacing 40.2 Å (Found : C, 77.2; H, 13.2%; equiv., 326. $C_{21}H_{44}O_2$ requires C, 77.2; H, 13.0%; equiv., 327).

The acid obtained from 20-methylheicosane-1 : 2-diol by oxidation with lead tetra-acetate, crystallised to purity from acetone and hexane, formed plates, m. p. and mixed m. p. 73—73.4°, crystal long spacing 40.4 Å (Found : C, 77.3; H, 12.9%; equiv., 325).

1 : 2-Diacetoxy-22-methyltricosane. The diacetate, b. p. 228—230°/1 mm., crystallised from methanol as needles, m. p. 37.5—38.5° (Found : C, 74.1; H, 12.0; Ac, 19. $C_{23}H_{54}O_4$ requires C, 74.0; H, 12.0; Ac, 18.9%).

22-Methyltricosane-1 : 2-diol. 1 : 2-Diacetoxy-22-methyltricosane was saponified and the diol crystallised from hexane and then methanol formed plates, m. p. 86.6—97°, crystal long spacing 52.3 Å (Found : C, 78.1; H, 13.6%; *M*, 360. $C_{24}H_{50}O_2$ requires C, 77.8; H, 13.6%; *M*, 371).

Synthesis of 21-methyldocosanoic acid. 18-Methylnonadecanoic acid (5.5 g., 0.0175 mole), ethyl hydrogen glutarate (4.4 g., 0.027 mole), and sodium (0.02 g., 0.0008 g. atom) were electrolysed, to yield 21-methyldocosanoic acid (0.75 g.), plates (from acetone and ethanol), m. p. 77.9—78.1°, crystal long spacing 43.9 Å (Found : C, 78.0; H, 13.2%; equiv., 353. $C_{23}H_{46}O_2$ requires C, 77.9; H, 13.1%; equiv., 355).

21-Methyldocosanoic acid, obtained from 22-methyltricosane-1 : 2-diol by oxidation with lead tetra-acetate and crystallised to purity from acetone and hexane, formed plates, m. p. and mixed m. p. 76—76.6°, crystal long spacing 43.9 Å (Found : C, 77.9; H, 13.0%; equiv., 354).

Octadec-1-ene. Octadecyl alcohol (27 g., 0.1 mole) and palmitic acid (30 g., 0.114 mole) were heated together at 240—260° until evolution of water had ceased (*ca.* 1 hr.), and then distilled (b. p. 350—360°/600 mm.). The crude octadecene was separated from the bulk of the palmitic acid by distillation at atmospheric pressure through a "Towers 10-plate" fractionating column, then fractionated in the spinning-band column, and the material of constant b. p. and refractive index was collected (8.8 g.; b. p. 129°/1 mm., n_D^{20} 1.4408 ± 0.0001. Cf. Wibaut, *Rec. Trav. chim.*, 1948, 67, 113).

(±)-n-1 : 2-Diacetoxyoctadecane. The method of Swern, Billen, and Scanlan (*loc. cit.*) was modified as follows : To a slowly stirred mixture of octadec-1-ene (16.3 g.) and formic acid (155 c.c.; 98—100%) at room temperature was added hydrogen peroxide (9.9 g. of 30% hydrogen peroxide, *i.e.*, 30% excess). The mixture was heated at 40° with slow stirring for 20 hr. The excess of formic acid was taken off under reduced pressure and the residue refluxed with potassium hydroxide (25 g.) in ethanol (200 c.c.) for 1 hr. The mixture was evaporated to small volume under reduced pressure, diluted with water, and extracted with ether. The ethereal layer was washed with water, dried (Na_2SO_4), and evaporated. The residue crystallised from pentane, to yield the crude diol (14.3 g.; m. p. 78.5—80.5°). It was dissolved in acetic anhydride (50 c.c.) and heated on a water-bath for 5 hr. The excess of acetic anhydride was removed and the residue fractionated at 1 mm. in the spinning-band column. The material of constant b. p. and refractive index (b. p. 191.5° ± 0.5°, n_D^{20} 1.4419 ± 0.0001) crystallised from methanol in needles, m. p. 39.5—40°, after resolidification m. p. 41.8—42.8° (Found : C, 71.5; H, 11.6; Ac, 23.3. Calc. for $C_{22}H_{42}O_4$: C, 71.3; H, 11.4; Ac, 23.2%). Niemann and Wagner (*J. Org. Chem.*, 1942, 7, 227) record m. p. 40.0° for (±)-1 : 2-diacetoxyoctadecane.

(±)-Octadecane-1:2-diol. Pure (±)-1:2-diacetoxyoctadecane was saponified and the diol crystallised from methanol as a colourless powder; m. p. 81—81·8°, crystal long spacing 40·4 Å (Found: C, 75·6; H, 13·5%; *M*, 285. Calc. for $C_{18}H_{38}O_2$: C, 75·5; H, 13·4%; *M*, 287). Swern *et al.* (*J. Amer. Chem. Soc.*, 1946, **68**, 1505) record m. p. 80—81° and Niemann and Wagner (*loc. cit.*); m. p. 79·0—79·5° for (±)-octadecane-1:2-diol.

The authors thank Drs. W. S. Rapson and D. A. Sutton for many helpful discussions, Mr. F. W. G. Schöning for the microanalyses, Dr. J. N. van Niekerk and Mr. E. R. L. Schöning for the X-ray measurements, Mr. C. A. J. Hoeve for the infra-red absorption spectra, and Prof. Cason for a sample of 17-methyloctadecanoic acid. This paper is published by permission of the South African Council for Scientific and Industrial Research. Acknowledgment is made of support from the South African Wool Board.

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[Received, June 15th, 1953.]

(85 g.) in ethanol (1 l. of 96%). The mixture was stirred at 40° for 24 hr. To the saponified mixture was added ethanol (660 c.c.) and water (1000 c.c.). The solution was shaken three times in a separating funnel (10 l.) with *n*-hexane. The combined hexane extracts, after being kept overnight in a tall cylindrical separating funnel, were separated from the sediment and shaken with 60% ethanol (2 × 500 c.c.). Evaporation of the hexane solution yielded extract I (131 g.).

The above alcohol solutions were combined and extracted in a continuous liquid-liquid extractor with hexane for 24 hr. The hexane extract was washed with 60% ethanol (200 c.c.) and evaporated to yield extract II (59 g.). The alcoholic wash-liquors were added to the next batch of saponified wax. The total yield of unsaponifiable material (extracts I and II) was 190 g. (38% of the wax).

Chromatographic Preparation of the Diols.—The wool-wax unsaponifiable material (500 g.), dissolved in wet ether (8 l., saturated with water at 6°), was filtered through a column of alumina (Peter Spence, type H), reactivated by 24 hr.' heating, with occasional stirring, at 400° (5 kg.; 30-cm. in height). The column was eluted with wet ether (24 l.), followed by ether (20 l.) containing ethanol (1% of 75%). The eluate was collected in 2-l. fractions. Those containing the material most strongly adsorbed were analysed by Karnovsky and Rapson's periodate titration method (*J. Soc. Chem. Ind.*, 1946, **65**, 138), and those containing considerable quantities of diols were amalgamated.

In this way, a crude diol fraction (186 g.) was obtained from the unsaponifiable material (2.2 kg.). It crystallised from ether and ethanol as a white powder (107 g.). The mother-liquors and fractions containing low diol contents (200 g.) were combined and rechromatographed. The crude diol fraction so obtained after crystallisation yielded further diol (30 g.). The total yield of crude diols (137 g.) was 6.2% of the unsaponifiable material.

To separate the diols from long-chain alcohols and other impurities, they were acetylated and distilled at 1 mm. in several batches in an electrically heated and vacuum-jacketed fractionating column (12 mm. internal diam.; 20 cm. long) packed with $\frac{1}{8}$ " Dixon gauze rings at a reflux ratio of about 1 : 10. The fractions of similar b. p. and *n* were combined to form the four following fractions: (1) b. p. 190—200°, n_D^{30} 1.4445, 54 c.c.; (2) b. p. 220—225°, n_D^{30} 1.4510, 37 c.c.; (3) b. p. 225—250°, solid, 32 c.c.; (4) b. p. 250—290°, n_D^{30} 1.4500—1.151, 59 c.c.

Fractions 1, 2, and 3 were separately saponified and crystallised from ether. Fraction 4, which contained the bulk of the high-boiling alcohols, was saponified and rechromatographed and the diol fraction crystallised. The material, obtained from the mother-liquors after crystallisation of the diols (fractions 1, 2, 3, and 4), was chromatographed and the diol fraction was obtained by crystallisation. A total of 90 g. of purified diols was obtained (4% yield). The above five diols fractions were separately acetylated and distilled. Distillates of similar b. p. were combined and redistilled where necessary.

The distillation was carried out in the microspinning band fractionating column of Piro and Glover (U.S. Pat. 2,608,528; *Chem. Abs.*, 1952, **46**, 9899; supplied by Messrs. H. S. Martin and Co., Illinois, U.S.A.) after modification as follows: (1) The pot heater, because of poor heat transfer, was replaced by two separately controlled heaters, one for the pot and the other for the exposed part of the column above the pot. (2) The column heater, because of uneven heating, was replaced by two evenly wound spiral heating elements. This allowed a better control over the temperature gradient in the column. (3) The partial condenser was replaced with a hollow centrally placed cold-finger condenser, which was fashioned at the end in the shape of hollow cone drawn down at one side to form a dripper. With this device it was possible, by comparison of the drop rate from this condenser with that from the dripper at the base of the column, to maintain a fine control over the column equilibrium. (4) The receiver was replaced by an electrically warmed one of the Perkin triangle type, which could be evacuated by means of a separate vacuum pump in order to avoid disturbing the column pressure. This type of still has a relatively low hold-up and low pressure drop and is thus suited to the fractionation of small quantities of not highly stable materials. The column temperatures were measured by means of iron-constantan thermocouples and recorded on a recording potentiometer (Messrs. Brown Instrument Co., Philadelphia, U.S.A.). The manostat of Birch *et al.* (*J. Soc. Chem. Ind.*, 1947, **66**, 3340) was used to maintain a pressure of 1 mm. with a maximum variation of about 0.02 mm. or a head-temperature variation of \pm about 0.25°.

For the distillation, the column was operated at a boil-up rate of 30—40 c.c./hr., pressure drop of 1.2—1.5 mm., and rotor speed of 1200 r.p.m. Under these conditions of operation, it had an efficiency of about 15 theoretical plates when tested with Williams's mixture (*Ind. Eng. Chem.*, 1947, **39**, 779). This is much lower than that claimed by the makers for operation at

atmospheric pressure (*viz.*, 100 theor. plates). It is possible that the value is low because this test mixture does not wet glass well (cf. Benner, Dinardo, and Tobin, *Ind. Eng. Chem.*, 1951, **43**, 722; and Williamson, *J. Appl. Chem.*, 1951, **1**, 33). Comparison of our distillation curves with those of Murray (*J. Amer. Oil Chem. Soc.*, 1951, **28**, 1) suggests that our column is of roughly equal efficiency.

Isolation of the Pure 1 : 2-Diols.—*n*-Hexadecane-1 - 2-diol. A portion of the material from the C₁₆ plateau (b. p. 173°/1 mm.) was saponified with alcoholic potassium hydroxide. Water was added and the diol, extracted with ether, was washed with water and dried (Na₂SO₄). After removal of the ether, the remaining diol was crystallised to purity from hexane and methanol, forming plates, m. p. 84—84.4°, crystal long spacing 36.7 Å (Found: C, 74.4; H, 13.4%; M, 261. C₁₆H₃₄O₂ requires C, 74.4; H, 13.3%; M, 258).

The molecular weight was determined by the volumetric method of Karnovsky and Rapson (*loc. cit.*). The m. p. was not depressed on admixture with an authentic sample of hexadecane-1 : 2-diol, m. p. 84—84.4°, prepared from (–)-*n*-2-hydroxyhexadecanoic acid by reduction with lithium aluminium hydride (Horn, Hougen, and von Rudloff, *Chem. and Ind.*, *loc. cit.*).

n-Pentadecanoic acid. To hexadecane-1 : 2-diol (1 g.) in dry benzene at 50—60° was added slowly a solution of lead tetra-acetate (4 g.; Mendel, *Rec. Trav. chim.*, 1940, **59**, 720) in dry benzene (100 c.c.) and acetic acid (a few drops). A stream of air was passed through the mixture during the addition, and for 2 hr. thereafter. The mixture was then poured into an equal volume of dilute sulphuric acid, shaken, and centrifuged. The benzene layer was extracted several times with concentrated sodium hydroxide solution. The combined aqueous layers were extracted with ether, and acidified, and the liberated acid was taken up in ether. The ethereal solution was washed with water, dried (Na₂SO₄) and evaporated. The acid crystallised from pentane in plates, m. p. 52—52.2° (0.75 g.) (Found: C, 74.1; H, 12.6%; equiv., 242. Calc. for C₁₅H₃₀O₂: C, 74.3; H, 12.5%; equiv., 242.4). There was no m. p. depression on admixture with an authentic sample of pentadecanoic acid prepared from (±)-*n*-2-hydroxyhexadecanoic acid (Mendel and Coops, *Rec. Trav. chim.*, 1939, **58**, 1140), m. p. 52.2—52.5° (Found: C, 74.5; H, 12.7%; equiv., 243). Francis, Piper, and Malkin (*Proc. Roy. Soc.*, 1930, **128**, A, 214) record m. p. 52.1°.

1 : 2-Diacetoxy-16-methylheptadecane. The diacetate from the C₁₈ plateau (b. p. 186.5°/1 mm.) crystallised from methanol at –20° as needles, m. p. 16—17°, [α]_D²⁰ = +0.012° (no solvent) (Found: C, 71.2; H, 11.6; Ac, 23.1. C₂₂H₄₂O₄ requires C, 71.3; H, 11.4; 2Ac, 23.2%).

16-Methylheptadecane-1 : 2-diol. 1 : 2-Diacetoxy-16-methylheptadecane was saponified and the diol crystallised from methanol and hexane as plates m. p. 75—75.4°, crystal long spacing 40.6 Å (Found: C, 75.8; H, 13.4%; M, 288. C₁₈H₃₈O₂ requires C, 75.5; H, 13.4%; M, 287). It showed no m. p. depression on admixture with 16-methylheptadecane-1 : 2-diol obtained by lithium aluminium anhydride reduction of (–)-16-methyl-2-hydroxyheptadecanoic acid (Horn, Hougen, and von Rudloff, unpublished work).

15-Methylhexadecanoic acid. 16-Methylheptadecane-1 : 2-diol was oxidised with lead tetraacetate as described above. The acid was crystallised to purity from acetone, hexane, and methanol, forming plates, m. p. 60.1—60.5°, crystal long spacing 33.3 Å (Ställberg, Stenhagen, *et al.*, *Arkiv Kemi, Min., Geol.*, 1948, **26**, A, No. 19, give 33.35 Å) (Found: C, 75.6; H, 12.7%; equiv., 271. Calc. for C₁₇H₃₄O₂: C, 75.5; H, 12.7%; equiv., 270). The m. p. showed no depression on admixture with an authentic specimen of 15-methylhexadecanoic acid, m. p. 60.3—60.5° (*idem*, *ibid.*).

1 : 2-Diacetoxy-18-methylnonadecane. The diacetate from the C₂₀ plateau (b. p. 203°/1 mm.) crystallised from methanol as needles, m. p. 26—27° (Found: C, 72.6; H, 11.7; Ac, 21.9. C₂₄H₄₆O₄ requires C, 72.3; H, 11.6; Ac, 21.6%).

18-Methylnonadecane-1 : 2-diol. The foregoing diacetate was saponified. The diol crystallised from hexane and methanol as plates, m. p. 80.8—81.1°, crystal long spacing 44.5 Å (Found: C, 76.6; H, 13.6%; M, 322. C₂₀H₄₂O₂ requires C, 76.4; H, 13.5%; M, 315).

17-Methyloctadecanoic acid. 18-Methylnonadecane-1 : 2-diol was oxidised with lead tetraacetate. The acid so obtained was crystallised to purity from hexane and acetone, giving plates, m. p. 66.6—66.8°, crystal long spacing 36.8 Å (Velick, *J. Amer. Chem. Soc.*, 1947, **69**, 2317, gives 36.2 Å) (Found: C, 76.5; H, 13.0%; equiv., 295. Calc. for C₁₉H₃₈O₂: C, 76.5; H, 12.8%; equiv., 298). The m. p. showed no depression on admixture with 17-methyloctadecanoic acid, m. p. 66.9—67.3°, kindly supplied by Prof. Cason.

1 : 2-Diacetoxy-20-methylheneicosane. The diacetate, b. p. 217—219°/1 mm., crystallised from methanol as needles, m. p. 33.3—33.8° (Found: C, 73.2; H, 11.9; Ac, 20.4. C₂₈H₅₀O₄ requires C, 73.2; H, 11.8; Ac, 20.2%).

16. Wool Wax. Part III.* *Synthesis of Some iso-Acids.*

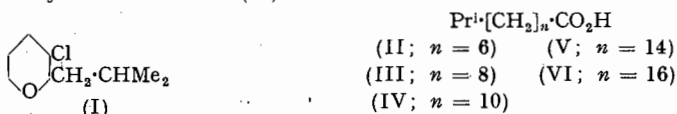
By F. W. HOUGEN, D. ILSE, DONALD A. SUTTON, and J. P. DE VILLIERS.

8 - Methylnonanoic,† 10 - methylundecanoic, 12 - methyltridecanoic, 16-methylheptadecanoic, and 18-methylnonadecanoic acids have been synthesised. The melting points and long X-ray crystal spacings of the solid acids and amides have been recorded for comparison with those of *iso*-acids from wool wax.

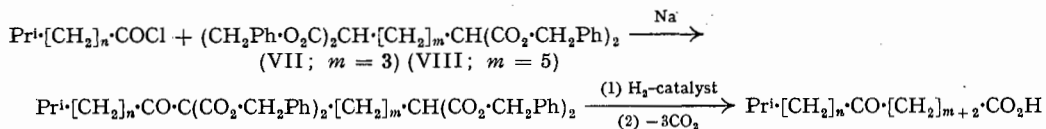
A SERIES of *iso*-acids was required in connection with the branched-chain compounds which exist in both the acid (Weitkamp, *J. Amer. Chem. Soc.*, 1945, **67**, 447) and alcoholic (Murray and Hatt, personal communication) fractions of saponified wool wax. We investigated four modern methods of chain lengthening for convenience of preparation of reasonable quantities of the necessary pure saturated *iso*-compounds. Some of the difficulties encountered since this work was initiated three years ago have been noted by others.

14-Methylpentadecanoic acid was not prepared since it has been satisfactorily identified in wool wax (Weitkamp, *loc. cit.*).

The dihydropyran method (Crombie and Harper, *J.*, 1950, 1707; Brandon, Derfer, and Boord, *J. Amer. Chem. Soc.*, 1950, **72**, 2120) was used with *isobutyl*magnesium bromide, giving *cis*- and *trans*-2-*isobutyl*-3-chlorotetrahydropyran (I), which on ring scission and hydrogenation gave 7-methyloctanol, converted through the bromide and nitrile into 8-methylnonanoic acid (II).



10-Methylundecanoic acid (III) was prepared from two sets of intermediates by a modification of Bowman's ketone method (*J.*, 1950, 174) in that the acid chlorides (4-methylpentanoylchloride, 2-methylpropanoyl chloride) reacted in the presence of one equivalent of sodium with polymethylene $\alpha\omega$ -dimalonic esters [benzyl pentane-1 : 1 : 5 : 5-tetracarboxylate (VII), benzyl heptane-1 : 1 : 7 : 7-tetracarboxylate (VIII)] instead of with monomalonic esters.

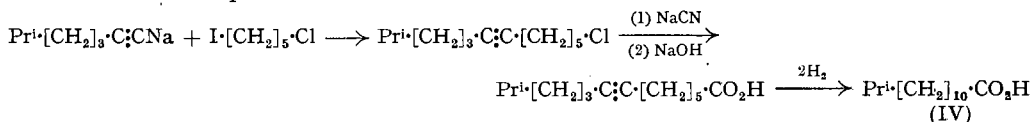


Yields of the keto-acids after debenzylation were poor so that the advantage of using the more readily available dimalonic esters is outweighed by losses probably arising, in part, from the occurrence of addition of acid chloride to both functional ends of the dimalonic ester molecule; in agreement with Nunn's report (*J.*, 1951, 1740) undesired ketonic hydrolysis of the intermediate β -keto-esters also occurred. A third source of loss in the case of (VII) arose from Dieckmann cyclisation, in the presence of the sodium, detected by the identification of *cyclohexanone* at the end of the series of operations; the use of malonic esters is therefore best restricted to those in which the chain is either too long or too short to favour intramolecular cyclisation. The keto-acids obtained after the hydrolysis step were smoothly reduced by Huang-Minlon's procedure (*J. Amer. Chem. Soc.*, 1946, **68**, 2487).

* Part II, Rossouw and von Rudloff (*J. Appl. Chem.*, 1952, **2**, 335).

† Geneva nomenclature ($\text{CO}_2\text{H} = 1$) is used throughout this paper for naming the *iso*-acids.

12-Methyltridecanoic acid (IV) was prepared by the chloro-iodide-alkylacetylene method of Strong *et al.* (*ibid.*, 1948, **70**, 1699, 3391) starting from 6-methylhept-1-yne and 1-chloro-5-iodopentane:



No difficulties were encountered, but attempts to condense 6-methylhept-1-yne with 1-chloro-10-iododecane were abortive. This is analogous to the failure of Taylor and Strong (*ibid.*, 1950, **72**, 4263) to condense alkylacetylenes containing more than nine methylene groups with chloro-iodides. Huber (*ibid.*, 1951, **73**, 2730) has since caused hept-1-yne to react with 1-chloro-10-iododecane, and Lumb and Smith (*Chem. and Ind.*, 1952, 358) have condensed the lithium derivative of *n*-tridec-1-yne with 1-chloro-3-iodopropane so that the limitations noted by Taylor and Strong and by us can be overcome at least in some cases. In practice the use of chloro-iodides is restricted by the difficulty of separating, by fractional distillation, dichloride-chloro-iodide-di-iodide mixtures when the chain is long.

The anodic method of Linstead *et al.* (*J.*, 1950, 3326) was used to synthesise 8-methylnonanoic acid (II) (from 4-methylpentanoic acid and ethyl hydrogen adipate), 10-methylundecanoic acid (III) (from 3-methylbutanoic acid and ethyl hydrogen azelate), 12-methyltridecanoic acid (IV) (from 4-methylpentanoic acid and ethyl hydrogen sebacate), 16-methylheptadecanoic acid (V) (from 12-methyltridecanoic acid and ethyl hydrogen adipate), and 18-methylnonadecanoic acid (VI) (from 10-methylundecanoic acid and ethyl hydrogen sebacate). This method proved the best, for our purpose, because of the ready availability of the starting materials, the reasonable yields obtained, and the smallness of the working losses arising from the fact that it is a one-step synthesis.

In both the Bowman and the Linstead method, it has been advisable to choose the starting materials so that the unwanted dicarboxylic acids arising from ketonic hydrolysis and symmetrical coupling are readily separable by fractional distillation from the desired products. To a lesser extent, dicarboxylic acid by-products also arise in the acetylenic method in cases where the chloro-iodide cannot be fractionated entirely free from di-iodide. It is suggested that, in such cases, the use of Craig's counter-current distribution method (*Ann. N.Y. Acad. Sci.*, 1951, **53**, 1015) is appropriate for purification of the end product since there are large differences in solubility in polar and non-polar solvents between aliphatic monocarboxylic and dicarboxylic acids of similar boiling points. Two examples are in the Experimental.

The properties of the synthetic acids and their amides are compared in the Table with those which Weitkamp (*loc. cit.*) and Velick (*J. Amer. Chem. Soc.*, 1947, **69**, 2317) recorded for wool wax *iso*-acids and with those taken from the compilation in the paper of Ställberg,

Acid *	M. p.		Long crystal spacing (Å)	
	Acid	Amide	Acid	Amide
8-Methylnonanoic	23.7—24.0° Liquid ⁴	103.8° 103.1 ¹	19.93 —	19.80 ^c 19.9 ²
10-Methylundecanoic	41.4—41.5 41.2 ¹	108.1—108.4 ^b 108 ¹	23.38 —	24.13 ^b 24.1 ²
12-Methyltridecanoic	53.3—53.6 53.4—53.6 ^a 53.3 ¹	108.1—108.3 ^a 107.3 ¹	26.82 26.80 ^a 26.8, ² 27.0 ³	— 27.14 ^a 27.0, ² 26.7 ³
16-Methylheptadecanoic	53.3—53.6 ³ 69.5—69.7 69.5 ¹	107.8—107.9 107.3 ¹	33.75 33.8 ^{2,3}	34.7 35.0, ² 34.5 ³
18-Methylnonadecanoic	75.3—75.6 75.3 ¹	105.9—106.1 105.1 ¹	37.52 37.8 ²	36.70 36.8 ²

* Specimens were prepared by the anodic method, unless indicated as ^a (Strong), ^b (Bowman), or ^c (Crombie and Harper).

¹ Weitkamp (*loc. cit.*). ² Velick (*loc. cit.*). ³ Ställberg and Stenhagen (*loc. cit.*). ⁴ Nelson and Dawson (*J. Amer. Chem. Soc.*, 1923, **45**, 2179).

Stenhagen, *et al.* (*Arkiv Kemi, Min., Geol.*, 1948, 26, A, No. 19). Direct comparison of our synthetic C_{12} and C_{20} *iso*-acids with specimens isolated by Dr. Weitkamp from wool wax showed that there was no depression of melting points; this and the close comparisons of properties in the Table confirm the validity of Weitkamp's structural assignments.

The long *X*-ray spacings were measured on a Philips high-angle Geiger counter spectrometer previously calibrated with a silicon standard. Filtered $Cu-K_{\alpha}$ radiation was used ($\lambda = 1.541 \text{ \AA}$). The specimens were prepared by melting them between two microscope slides and allowing them to cool under slight pressure. For every specimen it was possible to observe at least ten orders of "reflection."

EXPERIMENTAL

M. p.s are corrected.

Anodic Syntheses.—These were carried out under the conditions described under "Method B1" (Linstead *et al.*, *loc. cit.*, p. 3328) with dry methanol as solvent.

Craig Counter-current Distribution Separation of a Mono- and Di-carboxylic Acid Mixture.—Pimelic acid (0.15 g.) and 10-methylundecanoic acid (0.15 g.) were introduced into tube No. 0 of a 25-tube steel counter-current apparatus containing a two-phase solvent system made up from hexane (100), acetone (25), ethanol (13), and water (62) (figures in parentheses denote parts by volume). After 24 transfers had been applied the contents of the tubes were evaporated, giving pimelic acid, m. p. 105–106°, in tubes 0–4 inclusive (peak in tube 1) and 10-methylundecanoic acid, m. p. 40–41°, in tubes 20–24 inclusive (peak in tube 23).

8-Methylnonanoic acid (II).—(a) *From cis- and trans-2-isobutyl-3-chlorotetrahydropyran.* A mixture of *cis*- and *trans*-isobutyl-3-chlorotetrahydropyrans (11.8 g., 0.067 mole), prepared by the reaction of isobutylmagnesium bromide with 2 : 3-dichlorotetrahydropyran, was treated with powdered sodium (5 g., 0.218 g.-atom) in dry ether following the procedure of Crombie and Harper (*loc. cit.*); the product was poured into water and extracted with ether, to yield after distillation 7-methyloct-4-en-1-ol (5.5 g., 58% based on the 2-alkyl-3-chlorotetrahydropyran), b. p. 107°/21 mm. (Found: C, 75.8; H, 12.8. Calc. for $C_9H_{18}O$: C, 76.0; H, 12.8%). Hydrogenation afforded 7-methyloctanol (Found: C, 74.6; H, 14.0. Calc. for $C_9H_{20}O$: C, 74.9; H, 14.0%), characterised as its phenylurethane, m. p. 65° (Cason, *J. Amer. Chem. Soc.*, 1942, 64, 1102, records m. p. 65°). The 7-methyloctanol was converted into the bromide (3.5 g.; b. p. 107°/27 mm.) (Found: Br, 38.6. Calc. for $C_9H_{19}Br$: Br, 38.6%) with hydrogen bromide and thence through the nitrile into 8-methylnonanoic acid [cf. the preparation of (IV), method (a)] (1.03 g., 9% overall yield) (Found: C, 69.7; H, 11.7%; equiv., 173. Calc. for $C_{10}H_{20}O_2$: C, 69.5; H, 11.8%; equiv., 172.3). The amide had m. p. 103.8° (from acetone) (Found: C, 69.6; H, 12.7; N, 7.9. Calc. for $C_{10}H_{21}ON$: C, 70.1; H, 12.4; N, 8.2%).

(b) *From 4-methylpentanoic acid and ethyl hydrogen adipate.* The anodic method was used: 4-methylpentanoic acid (11.6 g., 0.1 mole), ethyl hydrogen adipate (33.6 g., 0.2 mole), and sodium (0.14 g., 0.006 g.-atom) were electrolysed to yield 8-methylnonanoic acid (6.58 g., 38% based on the 4-methylpentanoic acid), b. p. 106°/1 mm., f. p. 22.6° (from f. p. curve); after three recrystallisations from hexane at -50° it had m. p. 23.7–24.0°.

10-Methylundecanoic Acid (III).—(a) *From 4-methylpentanoyl chloride and benzyl pentane-1 : 1 : 5 : 5-tetracarboxylate.* Ethyl pentane-1 : 1 : 5 : 5-tetracarboxylate (36 g., 0.1 mole) was heated under reflux in dry benzene (150 c.c.) with powdered sodium (2.3 g., 0.1 g.-atom) until all the latter had dissolved (6 hr.). Benzyl alcohol (43.2 g., 0.4 mole) was added and distillation effected through a Fenske-helices-packed column until the theoretical quantity of ethanol (0.4 mol.) had been removed as the benzene-ethanol azeotrope (5 hr.) (cf. Bowman, *loc. cit.*, and Nunn, *loc. cit.*). 4-Methylpentanoyl chloride (13.45 g., 0.1 mole) was added slowly with stirring while cooling with ice-water, and the mixture was then refluxed (1 hr.) and cooled. Distilled water (250 c.c.) was added and the organic layer was taken up in ether, separated, washed (water), and dried (Na_2SO_4). The solvents were removed in a vacuum at $<40^\circ$, leaving a light yellow oil (70 g., 0.1 mole) which was shaken in dry ethanol-ethyl acetate (1 : 1; 200 c.c.) with hydrogen at 50 lb./sq. in. in the presence of palladium-calcium carbonate (10 g.; Pd, 1%); no hydrogen was absorbed; the catalyst was filtered off and replaced by the same amount of fresh catalyst which was filtered off after no hydrogen had been taken up; palladium-carbon (5 g.; Pd, 10%) was added and hydrogen was taken up (*ca.* 85% theor.) during 3 hours. The catalyst was filtered off and the solvents were removed in a vacuum; the residue was heated at 95° and then at 180° for a short period to complete the decarboxylation. The product was distilled through a short Vigreux column to give: (a) 6.65 g., b. p. 110°/25 mm., (b) 1.35 g.,

b. p. 110°/25 mm. to 85°/2 mm., (c) 1.8 g., b. p. 85—110°/2 mm., (d) 0.5 g., b. p. 127—135°/1 mm., (e) 5.3 g., b. p. 140—157°/1 mm., (f) 0.8 g., b. p. 157°/1 mm., (g) 1.3 g., b. p. 165—195°/1 mm. and (h) 1.3 g., b. p. 195—200°/1 mm. Fraction (a) contained cyclohexanone (2 : 4-dinitrophenylhydrazone, m. p. 157°; semicarbazone, m. p. 165°; and oxime m. p. 89°; mixed m. p.s gave no depression) and 4-methylpentanoic acid (*p*-bromophenacyl ester, m. p. 79°; amide m. p. 117°; mixed m. p.s gave no depression). Fractions (e) and (f) contained the required 7-keto-10-methylundecanoic acid; they were combined and reduced without purification by Huang-Minlon's procedure (*loc. cit.*) to give a product from which 10-methylundecanoic acid (1.0 g., 5%), m. p. 40—41°, was separated by fractional distillation (b. p. 130°/1 mm.) followed by crystallisation from aqueous methanol (Found : C, 71.6; H, 12.1%; equiv., 201. Calc. for C₁₂H₂₄O₂ : C, 72.0; H, 12.1%; equiv., 200.3); the amide was recrystallised from isohexane to m. p. 108.1—108.4° (Found : C, 72.7; H, 12.7. Calc. for C₁₂H₂₅ON : C, 72.4; H, 12.55%). Fraction (h) contained pimelic acid which was purified by crystallisation from aqueous hydrochloric acid (m. p. and mixed m. p. 105°).

(b) From 2-methylpropanoyl chloride and benzyl heptane-1 : 1 : 7 : 7-tetracarboxylate. By the above procedure but starting with 0.15 mole of ester with the appropriate amounts of sodium, benzyl alcohol, and the acid chloride, 10-methylundecanoic acid was obtained in 2 g. (6.7%) yield together with some azelaic acid.

(c) From 3-methylbutanoic acid and ethyl hydrogen azelate. The anodic method was used : 3-Methylbutanoic acid (88 g., 0.86 mole), ethyl hydrogen azelate (88 g., 0.41 mole), and sodium (0.6 g., 0.03 g.-atom) were electrolysed. 10-Methylundecanoic acid (37 g., 45% based on the ethyl hydrogen azelate) was recovered by distillation (b. p. 130°/1 mm.). After recrystallisation from 96% ethanol it had m. p. 41.4—41.5° and gave no depression of m. p. in admixture with the specimen supplied by Dr. Weitkamp.

12-Methyltridecanoic Acid (IV).—(a) From 6-methylhept-1-yne and 1-chloro-5-iodopentane. 6-Methylhept-1-yne (44 g., 0.4 mole) was added to sodamide (from 10 g., 0.435 g.-atom of sodium) in liquid ammonia and 1-chloro-5-iodopentane (93 g., 0.32 mole) was subsequently added (cf. Strong *et al.*, *loc. cit.*). After 24 hours the product was worked up, to yield 1-chloro-11-methyl-dodec-6-yne, b. p. 80°/1 mm. (24 g., 35% based on the 1-chloro-5-iodopentane), which was refluxed with ethanol (95%; 300 c.c.) for 72 hours with sodium cyanide (15 g., 0.31 mole); the precipitated sodium chloride was filtered off and the filtrate refluxed for 120 hours with sodium hydroxide (9.5 g., 0.24 mole) in water (50 c.c.). The 12-methyltridec-7-ynoic acid so produced was freed from non-acids by extraction of its alkaline solution (in 20% aqueous ethanol) with ether in the usual way and distilled, having b. p. 150—151°/1 mm. (11.8 g., 48% based on the 11-methyl-1-chlorododec-6-yne) [Found : C, 74.6; H, 10.6%; equiv., 219; 0.2598 g. took up 52.7 c.c. of H₂ (N.T.P.). C₁₄H₂₄O₂ requires C, 74.95; H, 10.8%; equiv., 224.3; 0.2598 g. is equivalent to 52.0 c.c. of H₂]; the acid had n_D^{20} 1.4556, d_4^{20} 0.91604, $[R_L]_D$ 66.5. (Calc. 66.4). Its *p*-bromophenacyl ester had m. p. 51° (from aqueous ethanol) (Found : C, 62.5; H, 6.95; Br, 19.0. C₂₂H₂₉BrO₂ requires C, 62.7; H, 6.9; Br, 19.0%). The acetylenic acid was smoothly hydrogenated in ethyl acetate in the presence of Adams's catalyst, to yield 12-methyltridecanoic acid, recrystallised from acetone and then hexane to m. p. 53.4—53.6°, b. p. 158°/2 mm. (Found : C, 73.6; H, 12.5%; equiv., 226. Calc. for C₁₄H₂₈O₂ : C, 73.6; H, 12.4%; equiv., 228.4), characterised as its amide, m. p. 108.1—108.3° (from hexane) (Found : C, 74.0; H, 12.6. C₁₄H₂₉ON requires C, 73.9; H, 12.85%).

(b) From 4-methylpentanoic acid and ethyl hydrogen sebacate. The anodic method was used : 4-methylpentanoic acid (23 g., 0.2 mole), ethyl hydrogen sebacate (46 g., 0.2 mole), and sodium (0.2 g., 0.009 g.-atom) were electrolysed, to yield 12-methyltridecanoic acid, recrystallised as above to give m. p. 53.3—53.6° (12 g., 27%) (Found : equiv., 225.5. Calc. for C₁₄H₂₈O₂ : equiv., 228.4).

16-Methylheptadecanoic Acid (V).—12-Methyltridecanoic acid (5 g., 0.022 mole), ethyl hydrogen adipate (16.8 g., 0.1 mole), and sodium (0.1 g., 0.004 g.-atom) were electrolysed, to yield 16-methylheptadecanoic acid (3 g., 47% based on the 12-methyltridecanoic acid) contaminated with sebacic acid. The latter was removed by counter-current distribution in a Craig apparatus with the two-phase solvent system detailed above; after 11 transfers the last two tubes contained the bulk of the 16-methylheptadecanoic acid, showing its preferential partition in the hydrocarbon layer; the acid had m. p. 68.0—68.7° and was recrystallised from acetone and then from hexane to m. p. 69.5—69.7° (Found : C, 75.8; H, 12.7%; equiv., 286. Calc. for C₁₈H₃₆O₂ : C, 76.0; H, 12.6%; equiv., 284.5). The amide had m. p. 107.8—107.9° (from acetone and hexane) (Found : N, 4.7. Calc. for C₁₈H₃₇ON : N, 4.9%).

18-Methylnonadecanoic Acid (VI).—10-Methylundecanoic acid (34 g., 0.17 mole), ethyl

hydrogen sebacate (113 g., 0.49 mole), and sodium (0.3 g., 0.01 g.-atom) were electrolysed, to yield 18-methylnonadecanoic acid (19 g., 36% based on the 10-methylundecanoic acid) (Found: C, 76.6; H, 12.9%; equiv., 312. Calc. for $C_{20}H_{40}O_2$: C, 76.7; H, 12.9%; equiv., 312.5), recrystallised from 96% ethanol to m. p. 75.3—75.6°, alone or mixed with the specimen supplied by Dr. Weitkamp. The amide had m. p. 105.9—106.1° (from acetone) (Found: N, 4.5. Calc. for $C_{20}H_{41}ON$: N, 4.5%).

The authors thank Dr. W. S. Rapson for suggesting this work, Dr. A. W. **Weitkamp** for the gift of 10-methylundecanoic and 18-methylnonadecanoic acid, and Mr. F. W. **G. Schöning** for carrying out the microanalyses. X-Ray measurements were made by Dr. J. N. van Niekerk and Mr. F. R. L. Schöning. This paper is published by permission of the South African Council for Scientific and Industrial Research.

NATIONAL CHEMICAL RESEARCH LABORATORY,
SOUTH AFRICAN COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH,
PRETORIA, SOUTH AFRICA. [Received, September 22nd, 1952.]

The Constitution of the Aliphatic Alcohols in Human Sebum

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(Received 9 August 1954)

Human sebum consists mainly of wax esters, free fatty acids, hydrocarbons and a small amount of glycerides. Whilst the free fatty acids have been investigated in detail (Weitkamp, Smiljanic & Rothman, 1947), little is known about the composition of the other fractions. The unsaponifiable matter has long been known to contain cholesterol (for review of earlier literature, see Čmelik, 1952) and squalene has recently been identified (Sobel, 1949; Mackenna, Wheatley & Wormall, 1952). By chromatographic studies these last authors found 14-20% of aliphatic alcohols in the unsaponifiable matter, but apart from an impure preparation of eicosanol (?) no individual alcohol was isolated.

The present paper describes an investigation into the aliphatic alcohols of human sebum. Sebum (surface skin 'fat') was obtained by extraction of hair from the heads of African (Bantu) males.

METHODS

Reversed-phase partition chromatography of fatty acids. The method of Howard & Martin (1950) (cf. Silk & Hahn, 1954) was extended to effect the resolution of six normal fatty acids ranging in chain length from six to eleven carbon atoms. It was found unnecessary for the present purpose to protect eluate and developing solvent against atmospheric CO_2 ; also, nitrogen was not purified before entering the titration vessel. The columns were maintained at tap-water temperature (18-22°). For loading of the columns, the acid mixtures were usually dissolved in the developing solvent. It was necessary to prepare a mull only when the mixed acids differed widely in chain length (such as C_5 - C_{12}).

The efficiency of separation is illustrated by the elution curve (Fig. 1) for a chromatogram of a synthetic mixture of pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, hendecanoic and dodecanoic acids (about 7 mg. of each). The C_5 and C_6 acids (unresolved) were eluted with water; C_7 with 10% (v/v) aqueous acetone, C_8 with 25%,

C₉ with 40%, C₁₀ with 45%, C₁₁ with 50% and C₁₂ with 55%. The eluate of this particular chromatogram was collected in 1.8 ml. samples and titrated with 0.02N alkali.

Isolation of total material forming complex with urea. The fraction to be treated with urea was dissolved in 96% (v/v) aqueous ethanol (5–10%, w/v, soln.). An amount of urea was added, equal to about 3 times the anticipated weight of complex-forming material in the fraction. The urea was dissolved by refluxing the solution. After standing overnight at room temperature, the solution was filtered and the precipitate was dried. A second precipitate of urea complex was sometimes obtained by treating the filtrate with more urea, or by further cooling or evaporation to a smaller volume of the filtrate. Complexes were recrystallized from ethanol in which urea (half the weight of the complex) was dissolved. Complexes were decomposed with water, and the alcohol fractions were recovered in ether. Residues which were left in the filtrates were recovered in the same way.

Fractional crystallization of urea complexes. This method was used for resolving mixtures which contained the acetates of a saturated normal, a saturated *iso* and an unsaturated normal alcohol, all having the same number of carbon atoms (cf. Tiedt & Truter, 1952). The prefix '*iso*' denotes a structure containing a terminal *isopropyl* group.

The material (about 10 g.) was dissolved (2%, w/v, soln.) in benzene containing 35% (v/v) of ethanol. Urea (in most cases 2 g.) was added to the solution and dissolved under reflux. After standing overnight at room temperature, the solution was filtered from the precipitated urea complex. The filtrate was again treated with urea (2 g.) to yield a second precipitate of urea complex. This procedure was continued until the precipitate consisted of urea only. The complexes were separately decomposed with water and the acetates recovered.

Chromatography on acid-washed alumina. Alumina (Peter Spence, Widnes, grade H) was washed with hot 2% (w/v) aqueous HNO₃ and then with warm water (distilled) and reactivated at 200°.

The unresolved mixture (about 0.5 g.) of an ω -hydroxy ester and an unhydroxylated ester dissolved in hexane

(5 ml.) was passed through a column (12 × 1 cm.) of acid-washed alumina (10 g.) and eluted successively with hexane (35 ml.), hexane containing 10% (v/v) of dry ether (40 ml.) and dry ether (80 ml.). The eluate was collected in 10 ml. fractions which were evaporated and weighed. The unhydroxylated ester was contained in the hexane eluate and the ω -hydroxy ester in the ether eluate.

Isolation of cholesterol through the oxalic acid complex. The method was essentially that of Pickard & Seymour (1945); the detailed procedure was as follows. The cholesterol-containing material was dissolved in anhydrous benzene (20%, w/v, soln.). Anhydrous oxalic acid (10% of the cholesterol-containing material) was added and the solution refluxed for 1 hr. The precipitated complex was filtered off the next day and washed with cold benzene. It was decomposed with ethanolic KOH solution and cholesterol was recovered in ether. The residual material was recovered in the same way.

Infrared absorption spectra. The spectra were recorded with a Perkin Elmer Infrared Spectrometer Model 12C; rock salt prism; path length, 0.5 mm. Alcohols (1.5%, w/v, in CCl₄) were examined in the 7.25 μ . region for the presence of *iso* and normal structures (Freeman, 1952). Unsaturated alcohols (1.5%, w/v, in CS₂) were examined in the 10.35 μ . region for *trans* configurations and in the 10.1 μ . region for terminal double bonds (Freeman, 1953).

EXPERIMENTAL AND RESULTS

Melting points are given corrected. They were measured in capillary tubes immersed in a paraffin bath which was electrically heated at a rate of 1°/6 min.

Extraction of sebum

Hair (28 kg. in batches of about 1.5 kg.) from Africans (Bantu) was obtained from the prison authorities. It was dried at 65° and 20 mm. Hg for 16 hr. and subsequently extracted continuously for 8 hr. with hot commercial 'isoheptane' in a stainless-steel percolator. The extracts were left to stand until the suspended solids settled. The

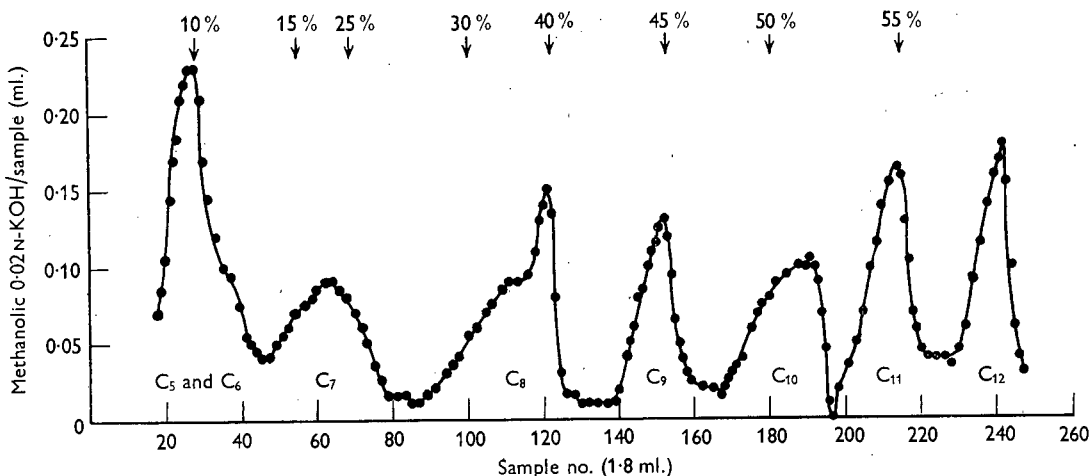


Fig. 1. Resolution of a mixture of pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, hendecanoic and dodecanoic acids (all *n* acids). Column 75 × 0.8 cm. Hold-up, 23 ml. Changes of solvent (% v/v, aqueous acetone) are indicated by the arrows.

solutions were siphoned off, the solids washed with 'isohexane' and the combined solutions evaporated to give crude sebum (1450 g.).

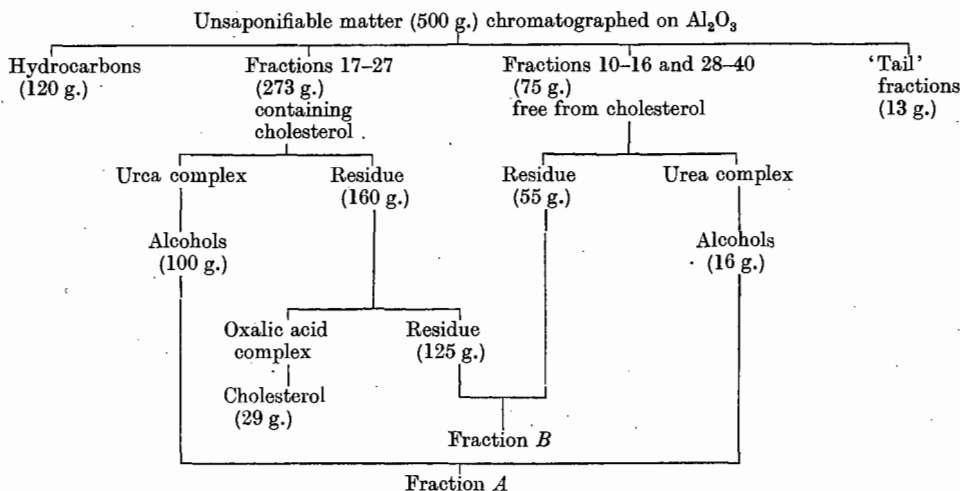
Separation of sebum into free acids, acids released from combination by saponification and unsaponifiable matter

A solution of crude sebum (1335 g.) in hexane (8 l.) was extracted in a separating funnel with 60% aqueous ethanol (4 l.) containing KOH (200 g.). The ethanolic extract, after being continuously extracted with hexane, was acidified (H_2SO_4) and the free acids (458 g.) were obtained by extraction with hexane. Further extraction of the ethanolic solution with ether gave a dark resinous material (24 g.). The

(41-44; 13 g.) which were eluted with ethanolic HCl were set aside. The remaining fractions were combined into a cholesterol-containing group (17-27; 273 g.) and a cholesterol-free group (10-16 and 28-40; 75 g.).

The cholesterol-containing (273 g.) and the cholesterol-free (75 g.) grouped fractions were separately treated with urea; aliphatic alcohols (100 and 16 g. respectively; subsequently combined and referred to as fraction A), and residual fractions (160 and 55 g. respectively) were obtained.

The residual fraction (160 g.) which contained cholesterol was treated with oxalic acid; cholesterol (29 g.) and a residual fraction (125 g.) were obtained. The latter was combined with the cholesterol-free residual fraction above (55 g.). This combined fraction is referred to as fraction B. These fractionations are shown in the diagram below:



combined hexane solutions which contained the neutral material were evaporated to a smaller volume (2 l.). Ethanol (2 l.) containing KOH (170 g.) was added and the mixture stirred for 24 hr. at 40°. Saponification was complete under these conditions. The acids released from combination (285 g.) were separated from the unsaponifiable matter (533 g.) by extraction of the latter with hexane from a solution of the saponified mixture in 60% (v/v) aqueous ethanol. A final continuous extraction (48 hr.) with hexane gave another portion of unsaponifiable matter (14 g.) which was not investigated further.

Separation of hydrocarbons, aliphatic alcohols and cholesterol from the unsaponifiable matter

The non-aromatic hydrocarbons were separated from the unsaponifiable matter by chromatography. Unsaponifiable matter (500 g.) in hexane (10 l.) was passed through a column (35 x 15 cm.) of alumina (Peter Spence, Type H; 5 kg.) and eluted with hexane (12.5 l.), ether (37.5 l.), ether containing 2% (v/v) of 96% (v/v) aqueous ethanol (15 l.), ether containing 10% of 96% ethanol (15 l.), 96% ethanol (10 l.), and finally 96% ethanol containing 10% (v/v) of aqueous 9.5N-HCl (10 l.). The eluate was collected in 2.5 l. fractions (44 in all), samples from which were evaporated to dryness and examined. The hydrocarbons (120 g.) were contained in the fractions (2-9) which were eluted with hexane and subsequently combined. The 'tail' fractions

Acetylation of fractions A and B

Fraction A (aliphatic alcohols; 116 g.) was heated with acetic anhydride (350 g.) on a water bath for 4 hr. The excess of solvent was distilled off under vacuum to give the acetates (133 g.). A portion of fraction B was similarly converted into acetates (30 g.).

Distillation of the acetylated fractions A and B

Two separate batches (30 and 103 g.) of the acetylated fraction A were distilled at 1 mm. Hg through a Pirots & Glover micro spinning-band column (cf. Horn & Hougén, 1953). The distillation curve for the larger batch is shown in Fig. 2 (curve A).

The acetylated portion of fraction B (30 g.) was distilled through the same column (Fig. 2, curve B). Only about half of this material distilled over below 240° mm.

Isolation of six saturated normal alcohols

By crystallizations (from methanol, ethanol or acetone, as suitable) of the appropriate distillation fractions (flats C_{14} , C_{16} , C_{18} , and fraction b of flat C_{20} ; Fig. 2), pure acetates of tetradecan-1-ol, hexadecan-1-ol, octadecan-1-ol, and eicosan-1-ol were obtained. Impure 1-tetracosanyl acetate was obtained by crystallization of fraction f of flat C_{24} . Fractional crystallization of urea complexes was applied to fraction d of flat C_{22} . The first two complexes obtained were recovered to

yield an impure preparation of 1-docosanyl acetate. The acetates were hydrolysed with ethanolic KOH and the recovered alcohols crystallized to purity (methanol, ethanol, hexane, ether). Analytical data for the alcohols and their acetates are given in Table 1.

Isolation of three saturated iso alcohols

Fractions *a*, *c* and *e* (Fig. 2), combined with the corresponding distillation fractions from the smaller batch (30 g.) of acetylated fraction *A*, were separately fractionated by crystallization of urea complexes. Fraction *a* (14 g.) was treated successively with 4×10 g. and 4×2 g. of urea. The acetate of 18-methylnonadecan-1-ol was recovered from the last (eighth) urea complex; the residue left after urea fractionation contained an unsaturated alcohol, as shown by its high iodine value, but this residue was not used as a source of eicos-10-en-1-ol since a better source (see below) was available. Fractions *c* (10 g.) and *e* (8 g.) were treated with 2 g. lots of urea and divided into 13 (numbered *c*1–*c*13) and 11 (numbered *e*1–*e*11) urea complexes respectively. Complexes *c*8 and *e*8 were decomposed to give the acetates of 20-methylheneicosan-1-ol and 22-methyltricosan-1-ol respectively. The acetates were hydrolysed and the recovered iso alcohols crystallized to purity. Analytical data are given in Table 1. The terminal isopropyl structure of the three iso alcohols was confirmed by their infrared absorption spectra which showed the characteristic splitting of the 7.25μ . band (Freeman, 1952). The isolated 18-methylnonadecan-1-ol and a synthetic specimen (Horn & Hougen, 1953) had identical infrared spectra.

Isolation and identification of three unsaturated normal alcohols

Eicos-10-en-1-ol. The single flat which was obtained by distillation of fraction *B* (Fig. 2) contained the acetate of an unsaturated C_{20} alcohol.

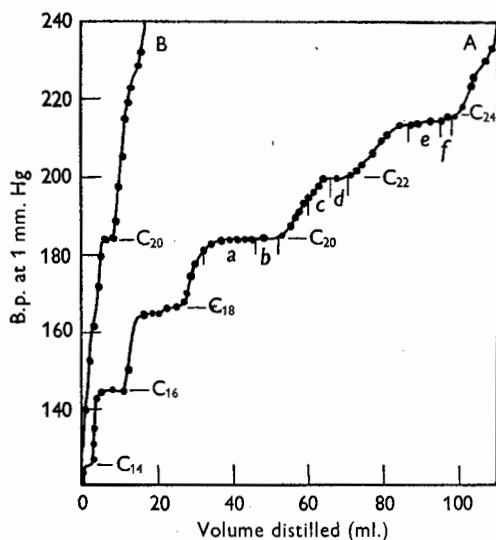


Fig. 2. Distillation curves (for conditions see text): *A*, acetates of the urea complex-forming material of sebum unsaponifiable matter (hydrocarbons excluded); *B*, acetates of the residual unsaponifiable matter.

A sample of the crude acetate was hydrogenated (Pd on $BaSO_4$ in glacial acetic acid) and took up 1.03 mol. prop. of H_2 (based on eicosenyl acetate). The hydrogenated sample melted at $39-41^\circ$. The acetate was saponified and the recovered alcohol crystallized from hexane and then methanol to m.p. $64.2-64.6^\circ$, unchanged on admixture with authentic eicosan-1-ol. (Found: C, 80.65; H, 14.4. Calc. for $C_{20}H_{42}O$: C, 80.5; H, 14.2%.)

The total fraction of eicosenyl acetate (about 2 g. from 30 g. of acetylated fraction *B*) was saponified, and the recovered alcohol (1.95 g.) crystallized from methanol at -60° to m.p. $23.5-24.4^\circ$ (analysis, see Table 1). Examination of the infrared spectra revealed that the double bond had the *cis* configuration and that there was no terminal double bond or terminal isopropyl group.

The alcohol (1.3 g.) was oxidized with performic acid in acetic anhydride according to the method of Fietelson (1950) for the oxidation of oleyl alcohol. The oxidation product was saponified to give crude trihydroxyeicosane (1.29 g.). Crystallization from hexane-methanol and from ether-methanol gave trihydroxyeicosane having m.p. $86.0-86.3^\circ$. (Found: C, 72.9; H, 12.9. $C_{20}H_{42}O_3$ requires C, 72.7; H, 12.8%.)

This product, combined with a less pure second crop from the mother liquors (1.0 g.), was oxidized in benzene solution with lead tetra-acetate and a stream of air according to Mendel & Coops (1939; cf. Horn & Hougen, 1953). The recovered acid reaction product (0.28 g.) was esterified with methanol- H_2SO_4 and the mixed esters (0.27 g.) were separated by chromatography on acid-washed alumina into unhydroxylated ester (0.089 g.) and ω -hydroxy ester (0.124 g.).

The unhydroxylated ester was hydrolysed to give the acid (0.080 g.). A portion of this acid (approx. 9 mg.) was examined by reversed-phase partition chromatography, and a single peak was obtained with no traces of neighbouring homologues. A second chromatogram was run with the unhydroxylated acid (4 mg.) in admixture with authentic decanoic acid (4 mg.). The total material was eluted in a single peak and the unhydroxylated acid was therefore decanoic acid.

The ω -hydroxy ester (0.124 g.) was saponified to give the acid (0.116 g.). The acid (in 40%, v/v, aqueous acetone) was passed through the chromatographic column in order to free it from any admixed unhydroxylated acid. The recovered acid was crystallized from hexane-benzene to m.p. $69-69.5^\circ$ (10-hydroxydecanoic acid has m.p. $75-76^\circ$ according to Chuit & Hausser, 1929). The acid was dissolved in aqueous alkali and oxidized with 5% (w/v) aqueous $KMnO_4$ at 50° . The oxidation product was worked up in ether, crystallized from water and extracted with boiling hexane to give a product having m.p. $127-129^\circ$ (raised to m.p. $130-132^\circ$ on admixture with sebacic acid of m.p. 133°). (Found: C, 59.8; H, 8.4. Calc. for $C_{10}H_{18}O_4$: C, 59.4; H, 9.0%.) Paper chromatograms of C_8 , C_9 and C_{10} dibasic acids, of the acid oxidation product and of this last in admixture with authentic sebacic acid, were run according to the method of Long, Quayle & Stedman (1951), except that the papers were sprayed with bromophenol blue and citric acid (Kennedy & Barker, 1951). The C_8 dibasic acid gave R_F , 0.46; C_9 acid, 0.51; C_{10} acid, 0.615; oxidation product, 0.575; a mixture of the oxidation product and C_{10} acid gave a single spot (R_F , 0.585). Although the sebacic acid obtained by the oxidation of the 10-hydroxydecanoic acid was not entirely pure, there can be no doubt about its identity.

SUMMARY

1. Twelve aliphatic alcohols of human sebum have been isolated and identified. They belong to three homologous series as follows: saturated normal alcohols (tetradecan-1-ol, hexadecan-1-ol, octadecan-1-ol, eicosan-1-ol, docosan-1-ol, tetracosan-1-ol), saturated *iso* alcohols (18-methylnonadecan-1-ol, 20-methylheneicosan-1-ol, 22-methyltricosan-1-ol) and unsaturated normal alcohols (eicos-10-en-1-ol, docos-12-en-1-ol, tetracos-14-en-1-ol).

2. The reversed-phase partition chromatographic technique for resolution of fatty acid mixtures (Howard & Martin, 1950) has been extended to include six shorter-chain acids having odd and even numbers of carbon atoms.

The author wishes to thank Dr W. S. Rapson for suggesting this work and Dr D. A. Sutton for helpful discussions. Dr D. H. S. Horn helped the author considerably with advice and guidance on many occasions and this is very gratefully acknowledged. The microanalyses were by Mr F. W. G. Schönig and Miss J. M. Theron, infrared absorption spectra by Mrs M. A. Raal and X-ray measurements by Dr J. N. van Niekerk. This paper is published by permission of the South African Council for Scientific and Industrial Research.

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the minimum percentages present is given in the last column of Table 1. The alcohols belong to three homologous series, namely: saturated normal alcohols (tetradecan-1-ol, hexadecan-1-ol, octadecan-1-ol, eicosan-1-ol, docosan-1-ol, tetracosan-1-ol), saturated *iso* alcohols (18-methylnonadecan-1-ol, 20-methylheneicosan-1-ol, 22-methyltricosan-1-ol) and unsaturated normal alcohols (eicos-10-en-1-ol, docos-12-en-1-ol, tetracos-14-en-1-ol).

Isolation of the mixed alcohols was effected by precipitation of their urea complexes from unsaponifiable matter that had previously been freed from non-aromatic hydrocarbons. Because the formation of urea complexes was not quantitative, a smaller fraction of alcohols was left in the residue. The mixed alcohols (as acetates) were separated by distillation into six fractions of increasing molecular weight (crude C_{14} , C_{16} , C_{18} , C_{20} , C_{22} and C_{24} alcohols). Each of the three lower-boiling fractions consisted of one saturated normal alcohol only. The three higher-boiling fractions were mixtures of normal, *iso* and unsaturated alcohols, which could not be resolved by distillation. These mixtures were separated by fractional crystallization of their urea complexes. One of the unsaturated alcohols (C_{20}) was present in appreciable amount in the residual unsaponifiable matter which did not form a urea complex, and was isolated from this fraction by distillation of the acetates; iodine values of the distillation fractions indicated that there were unsaturated alcohols present of chain lengths lower than C_{20} and higher than C_{24} , but the quantities were too small for their isolation. Some of these distillation fractions, however, deposited small amounts of crystals which in two of the fractions were most probably anthracene and chrysene (Hougen, 1954).

The saturated normal alcohols were identified by their melting points, elementary compositions, and X-ray long crystal spacings, and in some cases by acetyl values and the melting points and elementary compositions of the acetates. The *iso* alcohols were identified by their melting points, elementary compositions and infrared absorption spectra. There was sufficient material for measurement of the X-ray long crystal spacing of only one of the *iso* alcohols (C_{24}). This alcohol gave no clear X-ray pattern, and an impurity such as tetracosan-1-ol might therefore have been present in the sample. The unsaturated alcohols had one double bond and an unbranched chain as shown by their hydrogenation to the corresponding saturated normal alcohols. The double-bond positions were determined by oxidative cleavage of the alcohols into two acid fragments (ω -hydroxy acids and unhydroxylated acids) followed by the identification of these acids. The possibility was considered that the unsaturated alcohols were mixtures of double-bond

position isomers (cf. double-bond position isomers in the free fatty acids of sebum; Weitkamp *et al.* 1947), in which case the acid fission products would be homologous mixtures. The technique of reversed-phase partition chromatography of fatty acids (C_{12} - C_{18} acids (Howard & Martin, 1950); C_{20} - C_{24} acids (Silk & Hahn, 1954)) was extended to include six shorter-chain normal acids (C_6 - C_{11}); the unhydroxylated acid degradation products were analysed by this method. The C_{20} , C_{22} and C_{24} unsaturated alcohols each yielded decanoic acid with no traces of its homologues. In one case (degradation product from C_{24} alcohol) a small fraction of unidentified material appeared in the eluate in front of the decanoic acid. This unidentified material was neither a homologue of decanoic acid nor was it 14-hydroxytetradecanoic acid, as was shown by its position in the elution curve and by the melting point of the recovered material. Although this material was not identified, its presence clearly does not influence the argument about the structure of the tetracosenol component. The identification of decanoic acid as a degradation product of the C_{20} , C_{22} and C_{24} alcohols proved their structures to be eicos-10-en-1-ol, docos-12-en-1-ol and tetracos-14-en-1-ol respectively. The corresponding derived ω -hydroxy acids (10-hydroxydecanoic, 12-hydroxydodecanoic and 14-hydroxytetradecanoic acids) proved difficult to purify by crystallization. It was considered sufficient to identify one of them (10-hydroxydecanoic acid) by oxidation to the dibasic acid (sebacic acid).

The saturated normal alcohols of sebum are common constituents of natural waxes. The *iso* alcohols are apparently not of wide occurrence, but have been found in wool wax (Murray & Schoenfeld, 1952). The unsaturated alcohols have not been described before, but somewhat similar alcohols have been found in the seed oil of *Simmondsia chinensis* (eicos-11-enol and docos-13-enol; Green, Hilditch & Stainsby, 1936) and in sperm blubber oil (eicos-9-enol; Toyama, 1938).

In many natural waxes the acids and alcohols are structurally related, and this may suggest a common path in the biosynthesis of these two classes of compounds. Sheep sebaceous secretion (wool wax) is a striking illustration of this phenomenon as the acids and aliphatic alcohols belong to the same four homologous series of normal, *iso*, *ante-iso* and α -hydroxy compounds (Knol, 1954). A similar relationship does not exist between the alcohols and the free fatty acids of human sebum. Acids of odd- as well as even-numbered chain lengths are present, whilst *iso* acids have not been found (Weitkamp *et al.* 1947). The double bonds are differently situated in the acids and the alcohols. The 'combined' acids of sebum have not yet been reported upon in the literature.

Docos-12-en-1-ol. As already described, fraction *c* of distillation flat C_{22} (Fig. 2) was divided into thirteen urea complexes. The acetate which was recovered from complex *c*12 was hydrolysed to give an unsaturated C_{22} alcohol (see Table 1). The double bond in this alcohol had the *cis* configuration, as shown by its infrared absorption spectrum, and there was no terminal double bond or terminal *iso*-propyl group.

A sample of the alcohol was hydrogenated and took up 0.88 mol. prop. of hydrogen (based on docosenol). The recovered hydrogenated sample was crystallized from ether to m.p. 70.1–70.2°, unchanged on admixture with authentic *n*-docosenol. (Found: C, 81.0; H, 14.3. Calc. for $C_{22}H_{46}O$: C, 80.9; H, 14.2%.)

A preparation of docosenol (containing also some 20-methylheneicosanol) was obtained from the pooled urea complexes *c*11–*c*13 and the most pentane-soluble part of the recovered alcohols from complexes *c*9 and *c*10. This material (1.55 g.) was oxidized with performic acid and the product saponified to give *trihydroxydocosane* (1.27 g.). Crystallization from ethyl acetate, followed by many extractions with boiling hexane to remove monohydric alcohol, and one crystallization from ether, gave fine prisms, m.p. 87.4–88.1°. (Found: C, 73.9; H, 12.7. $C_{22}H_{46}O_3$ requires C, 73.7; H, 12.9%.)

This product was pooled with a second crop obtained from the mother liquors (total 0.66 g.) and oxidized with lead tetra-acetate and air. The recovered acid reaction product (0.52 g.) was converted into the methyl esters (0.43 g.), and the latter were chromatographed on acid-washed alumina to give unhydroxylated ester (0.15 g.) and ω -hydroxyester (0.2 g.).

The acid (0.093 g.) which was recovered from the unhydroxylated ester was examined by reversed-phase partition chromatography and found to be decanoic acid with no traces of neighbouring homologues. A mixed chromatogram with authentic decanoic acid confirmed this. The acid which was recovered from the first chromatogram was converted into its *p*-bromophenacyl ester, which was crystallized from aqueous ethanol to m.p. 65.1–65.4° (*p*-bromophenacyl decanoate has m.p. 66° according to Hopkin & Williams (1950)). Admixture with authentic *p*-bromophenacyl decanoate gave no depression of the melting point, whereas authentic *p*-bromophenacyl nonanoate depressed the melting point considerably.

The ω -hydroxy ester (0.2 g.) was crystallized from pentane to m.p. 33.1–33.8° (methyl 12-hydroxydodecanoate has m.p. 34–34.5° according to Chuit & Hausser (1929)). Chromatography of the ester on acid-washed alumina followed by crystallization from pentane failed to raise the melting point of the ester. The ester was saponified and the recovered ω -hydroxy acid crystallized (benzene, hexane–ethyl acetate) to m.p. 78.1–81.7° (12-hydroxydodecanoic acid, m.p. 84–85° (Chuit & Hausser, 1929)).

Tetracos-14-en-1-ol. As described before, fraction *e* of distillation flat C_{24} (Fig. 2) was divided into eleven urea complexes. The acetate contained in complex *e*10 was saponified to give an unsaturated C_{24} alcohol. The alcohol was crystallized (methanol, acetone, benzene) to m.p. 43.3–43.5° (see Table 1). The infrared absorption spectrum proved the double bond of the alcohol to have the *cis* configuration, and that no terminal double bond or *iso*-propyl group was present.

A sample of a less pure preparation of the alcohol (m.p. 41.6–42.0°) was hydrogenated and took up 1.06 mol. prop.

of hydrogen (based on tetracosenol). The hydrogenated sample was recovered and crystallized from ether to m.p. 75.1–75.3°, unchanged on admixture with authentic tetracosanol. (Found: C, 81.2; H, 14.4. Calc. for $C_{24}H_{50}O$: C, 81.3; H, 14.1%.)

A preparation (1.6 g.) of tetracosenol (containing some 22-methyltricosanol) was obtained from the pooled urea complexes *e*8–*e*11. The material was oxidized with performic acid and the product saponified to give *trihydroxytetracosane* (1.5 g.), crystallized from hexane, ether at –25° and methanol to m.p. 89–90.4°. (Found: C, 74.5; H, 13.1. $C_{24}H_{50}O_3$ requires C, 74.55; H, 13.0%.)

A less pure preparation of trihydroxytetracosane (0.95 g.) was oxidized with lead tetra-acetate and air, and the acid oxidation products (0.56 g.) were converted into the methyl esters (0.5 g.). The esters were chromatographed on acid-washed alumina to give unhydroxylated ester (0.15 g.) and ω -hydroxy ester (0.2 g.).

The unhydroxylated ester was hydrolysed to the acid (65 mg.) and the latter was examined by chromatography on the reversed-phase column. A small fraction of the total material (10–15%) was eluted with 45% acetone and the rest of the material with 50% acetone. The acid contained in the second peak was recovered and converted into its *p*-bromophenacyl ester, m.p. 65.3–65.5° (*p*-bromophenacyl decanoate, m.p. 66°) unchanged on admixture with an authentic specimen of *p*-bromophenacyl decanoate. (Found: Br, 21.5. Calc. for $C_{18}H_{26}O_3Br$: Br, 21.7%.) Confirmation that the unhydroxylated acid fraction was substantially decanoic acid was obtained by a chromatogram of this material admixed with authentic decanoic acid. The smaller fraction of the unhydroxylated acid material which was eluted in front of decanoic acid was recovered and melted at 40°.

The ω -hydroxy ester (0.2 g.) was crystallized from hexane to m.p. 41.7–43.0°. The ester was saponified and the recovered acid crystallized from hexane to m.p. 75–85.5° (14-hydroxytetradecanoic acid, m.p. 91–91.5°; Chuit & Hausser, 1929). (Found: C, 68.85; H, 11.6. Calc. for $C_{14}H_{28}O_3$: C, 68.8; H, 11.55%.) The acid in 45% aqueous acetone was passed through the reversed-phase column, but there was no improvement in the melting point of the acid recovered from the eluate.

DISCUSSION

The sebum was obtained by extraction of hair from a large number of people, and it might therefore have been contaminated with extraneous matter such as hair dressings, though these are not normally accessible to the subjects from whom the hair was obtained. Hair dressings consist mostly of neutral fats (glycerides) and mineral oils (cf. Weitkamp *et al.* 1947), and such materials do not yield a high percentage of long-chain alcohols on saponification. Contamination of the material reported upon here is therefore not likely to be appreciable in amount.

Twelve aliphatic alcohols of human sebum were identified. It is not possible to state with certainty the relative proportion of each present, since intermediate fractions and residues obtained during the lengthy fractionation contain unknown amounts of the various compounds; however, an indication of

Table 1. *Properties of the isolated alcohols and their acetates*

Calc. values for acetyl, C and H percentages are in brackets.

	Acetates				Alcohols					Minimum percentage present (g./100 g. non-saponifiables)
	M.p. (°)	Acetyl (%)	C (%)	H (%)	M.p. (°)	C (%)	H (%)	X-ray long crystal spacings (Å)		
Tetradecan-1-ol	13.4-14.0 14.0 ^a	17.0 (16.8)	74.6 (74.9)	12.6 (12.6)	37.7-38.0 38.26 ^g	78.2 (78.4)	14.0 (14.1)	— —	39.6 39.7 ^f	0.2 —
Hexadecan-1-ol	24.3-24.5 24.2 ^a	15.3 (15.1)	76.1 (76.0)	12.7 (12.8)	49.3-49.4 ^b 49.62 ^g	79.1 (79.3)	14.1 (14.1)	36.8 37.4 ^f	44.0 44.9 ^f	1.4 —
Octadecan-1-ol	32.7-32.9 32.8 ^a	13.9 (13.75)	77.05 (76.9)	13.0 (12.9)	58.0 ^b 57.98 ^g	79.9 (79.9)	14.15 (14.2)	41.2 41.3 ^f	— 50.2 ^f	2.5 —
Eicosan-1-ol	39.7-39.8 40 ± 0.5 ^a	13.0 (12.6)	77.5 (77.6)	13.0 (13.0)	65.05 65.5 ^g	80.4 (80.5)	14.2 (14.2)	45.4 —	— —	3.0 —
Docosan-1-ol	47.6-47.8 ^d 49-51 ^e	— —	— —	— —	70.3-70.4 70.6 ^g	81.0 (81.0)	14.1 (14.2)	49.8 49.95 ^f	— —	1.3 —
Tetracosan-1-ol	ca. 53.5 ^d 57 ^a	— —	— —	— —	75.5-75.7 74.8 ^g	81.3 (81.3)	14.2 (14.2)	53.2 54.0 ^f	— —	2.0 —
18-Methylnonadecan-1-ol	30.9-32.9 ^d	—	—	—	49.2-49.4 ^b 50.3 ^e	80.3 (80.5)	14.2 (14.2)	— —	— —	0.5 —
20-Methylheicosan-1-ol	—	—	—	—	55.4-55.9 57.1 ^e	80.9 (80.9)	14.3 (14.2)	— —	— —	0.4 —
22-Methyltricosan-1-ol	—	—	—	—	62.4-62.7 62.4 ^e	81.15 (81.3)	14.3 (14.2)	— —	— —	0.5 —
Eicos-10-en-1-ol	—	—	—	—	23.5-24.4	81.0 (81.0)	13.7 (13.6)	— —	— —	0.6 —
Docos-12-en-1-ol	—	—	—	—	—	81.4 (81.4)	13.8 (13.7)	— —	— —	0.4 —
Tetracos-14-en-1-ol	—	—	—	—	43.3-43.5	81.6 (81.7)	13.8 (13.7)	— —	— —	0.5 —

^a Phillips & Mumford (1934).^c Tiedt & Truter (1952).^e Murray & Schoenfeld (1952).^g Ralston (1948).^b The m.p. was unchanged by admixture with an authentic sample.^d The acetate was not obtained pure.^f Malkin (1930).

Wool Wax. Part V.* The Constitution of the
 α -Hydroxy-acids derived therefrom.

By D. H. S. HORN, F. W. HOUGEN, E. VON RUDLOFF, and D. A. SUTTON.

[Reprint Order No. 4552.]

The saponifiable fraction of wool wax from merino wool yields nearly 30% of a mixture of optically active 2-hydroxy-*n*-dodecanoic, -*n*-tetradecanoic, -*n*-hexadecanoic, -*n*-octadecanoic, and -16-methylheptadecanoic acid.

EARLIER investigations of the saponifiable fraction of wool wax (Lewkowitsch, *J. Soc. Chem. Ind.*, 1892, **11**, 134; 1896, **15**, 14; Darmstaedter and Lifschütz, *Ber.*, 1896, **29**, 1474, 2890; Röhmann, *Biochem. Z.*, 1916, **77**, 298; Abraham and Hilditch, *J. Soc. Chem. Ind.*, 1935, **54**, 398T; Bertram, *J. Amer. Oil Chem. Soc.*, 1949, **26**, 454) led to the view that it contains hydroxy-acids. Kuwata (*J. Amer. Chem. Soc.*, 1938, **60**, 559) isolated a 2-hydroxy-*n*-hexadecanoic acid in 7% yield, and Weitkamp (*ibid.*, 1945, **67**, 447) isolated also a 2-hydroxy-*n*-tetradecanoic acid in 0.2% yield.

Reduction, with lithium aluminium hydride, of a sample of the acids of wool wax extracted from merino wool and estimation of the 1 : 2-diols in the products with periodic acid indicated the presence of about 30% of α -hydroxy-acids in the original acid mixture. By solvent distribution, the mixture was separated into three broad groups (Figure) : a resinous, polar group of acids (11%) of high molecular weight, α -hydroxy-acids (27%), and unhydroxylated acids (62%).

As it was not possible to purify all the individual α -hydroxy-acids completely by solvent distribution with the 30-tube apparatus available, their methyl esters were first distilled, at 1 mm. pressure, in a micro-spinning-band fractionating column described previously (Part IV *). The pure components were isolated from the appropriate distillates by crystallisation, together, where necessary, with solvent distribution. The three pure α -hydroxy-acids obtained were identified, by oxidation with lead tetra-acetate in a current of air to the corresponding fatty acids containing one carbon atom less (cf. Mendel and Coops, *Rec. Trav. chim.*, 1939, **58**, 1140), as 2-hydroxy-*n*-dodecanoic, -*n*-tetradecanoic, and -*n*-hexadecanoic acid. The C_{18} ester fraction of the mixture is more thermolabile than the lower homologues and could not be resolved readily by fractional distillation. Also its physical properties were rather different from those of the other fractions and an infra-red study showed that it contained a large proportion of a branched-chain acid.

The wool wax acids were reduced with lithium aluminium hydride, and the 1 : 2-diols originating from the α -hydroxy-acids were separated in a yield of 22% by chromatography on active alumina. Distillation of the acetates, hydrolysis, and crystallisation of appropriate fractions yielded pure *n*-tetradecane-, *n*-hexadecane-, *n*-octadecane-, and

R in R·CH(OH)·CO ₂ H	Me ester,		Acid,			Diol,	
	m. p.	b. p./1 mm.	m. p.	$[\alpha]_D$ (CHCl ₃)	%	m. p.	diacetate, b. p.
C ₁₀ H ₂₁	24—25°	—	78.0—78.5°	-2.8°	2.2	—	—
C ₁₂ H ₂₅	34.8—35.0	133.5—136.5°	88.2—88.5 ^a	-3.1	13.5	77.6—77.9°	154.5°
C ₁₄ H ₂₉	45.5—45.7	153.5—154.0	93.3—93.5 ^b	-3.2	67.8	84.0—84.2	172
C ₁₆ H ₃₃	—	—	—	-3.8	3.3	87.7—87.9	185
CHMe ₂ ·C ₁₃ H ₂₈ ...	ca. 40°	—	ca. 78°	—	12.2	75.0—75.8	ca. 188

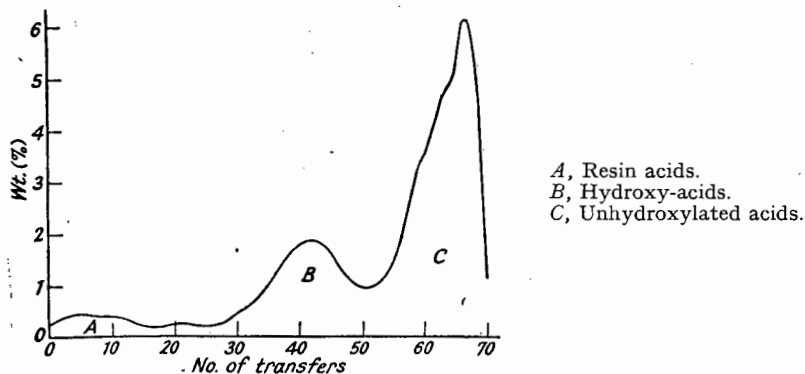
^a Weitkamp (*loc. cit.*) gives 88.2—88.5°, and Kuwata (*loc. cit.*) gives 86—87°. ^b Weitkamp (*loc. cit.*) gives 93.6°.

16-methylheptadecane-1 : 2-diol. After purification of the octadecanediol, too little remained for further characterisation by oxidation. However, the infra-red absorption is

* Part IV, *J.*, 1953, 3533.

identical with that of synthetic (\pm)-octadecane-1:2-diol. 16-Methylheptadecane-1:2-diol, on the other hand, showed the characteristic doublet in the 1360—1400-cm.⁻¹ range of the infra-red absorption spectrum (cf. Part IV). The material was further characterised by oxidation to 15-methylhexadecanoic acid, identical with a specimen prepared from 12-methyltridecanoic acid (Part III, *J.*, 1953, 98) by anodic coupling with the half ester of glutaric acid.

The two remaining α -hydroxy-acids in the mixture are thus characterised as 2-hydroxy-*n*-octadecanoic acid and -16-methylheptadecanoic acid. The properties of the compounds isolated are summarised in the Table



EXPERIMENTAL

Preparation of the Wool Wax Acids.—Saponification of wool wax extracted from merino wool and removal of the unsaponifiable material were carried out as described in Part IV (*loc. cit.*). The combined alcoholic solutions of the soluble potassium soaps were diluted with water and acidified and the fatty acids extracted with ether. After drying (Na_2SO_4), the ethereal extracts were evaporated to yield the main acid fraction. The yield (45—47 g. from 100 g. of wax) is about 90% of the acids present in the wax because the acids of low molecular weight which are very soluble in water and those with very high molecular weight which form potassium soaps insoluble in 60% ethanol were isolated separately.

Isolation of the Hydroxy-acid Fraction.—The α -hydroxy-acids were separated from the "resin acids" and the unhydroxylated acids by counter-current distribution of their methyl esters in an all-glass, 30-tube apparatus (Messrs. Otto Post & Co., New York, U.S.A.), each tube of which accommodated 100 c.c. of each phase. The methyl esters (30 g.) of the main acid fraction were introduced into the first three tubes and distributed between commercial hexane and 85% ethanol. Each fraction was separately evaporated and weighed (see Figure). The quantity of α -hydroxy-ester present in each fraction was determined by Hochstein's active hydrogen method (*J. Amer. Chem. Soc.*, 1949, **71**, 305). Those fractions which contained considerable quantities of hydroxy-esters were amalgamated (11.4 g.) and redistributed as before. In this way the esters were separated into three main groups: (a) those of high molecular weight, dark and polar methyl esters of the "resin acids" (3.3 g., 10.8%); (b) α -hydroxy-esters (8.2 g., 27.3%); and (c) unhydroxylated esters (18.6 g., 61.9%).

Separation of the α -Hydroxy-acids.—The material (4.5 g.) representing the peak of the second distribution curve was nearly pure methyl 2-hydroxy-*n*-hexadecanoate. Crystallised from ether and then from acetone, it formed a white powder (3.1 g.), m. p. 45.5—45.7° (Weitkamp, *loc. cit.*, records m. p. 45.6°), $[\alpha]_D -3.6^\circ$ (*c.* 10 in CHCl_3) (Found: C, 71.4; H, 12.0; active H, 0.72%; sap. equiv., 288. Calc. for $\text{C}_{17}\text{H}_{34}\text{O}_3$: C, 71.3; H, 12.0; active H, 0.70%; equiv., 286). Saponification yielded 2-hydroxy-*n*-hexadecanoic acid, a white powder (from acetone, then hexane), $[\alpha]_D +3.6^\circ$ (*c.* 6 in pyridine) (cf. Table) [Kuwata, *loc. cit.*, records $[\alpha]_D -1.0^\circ$ (*c.* 5.2 in EtOH)] (Found: C, 70.4; H, 11.9; active H, 0.75%; equiv., 272. Calc. for $\text{C}_{16}\text{H}_{32}\text{O}_3$: C, 70.5; H, 11.8; active H, 0.74%; equiv., 272). Oxidation of the acid with lead tetra-acetate (method: Mendel and Coops, *loc. cit.*) yielded pentadecanoic acid, m. p. 51.5—52.0° (Found: C, 74.5; H, 12.3%; equiv., 241. Calc. for $\text{C}_{15}\text{H}_{30}\text{O}_2$: C, 74.3; H, 12.5%; equiv. 242), which did not depress the m. p. of pentadecanoic acid (m. p. 52.0—52.5°) prepared by oxidation of synthetic (\pm)-2-hydroxy-*n*-hexadecanoic acid.

The remaining fractions from the second distribution, together with the mother-liquors from

the crystallisation of the methyl-2-hydroxy-*n*-hexadecanoate (altogether 5.1 g.), were re-distributed in a 90-transfer experiment, being separated into three well-defined groups. Analysis of the material from the tube at the middle of each group, after crystallisation, corresponded very closely to the values expected for methyl 2-hydroxytetradecanoate, 2-hydroxyhexadecanoate, and 2-hydroxyoctadecanoate, respectively. A value was assumed for the partition coefficient of methyl 2-hydroxydodecanoate (which did not form a well-defined peak in the distribution curve) based on the differences found between the coefficients of the other members (C_{14} , C_{16} , C_{18}) of the homologous series. Then, allowing for the quantity of 2-hydroxy-*n*-hexadecanoic acid removed by crystallisation, it was possible to calculate fairly accurately the proportions of the individual hydroxy-acids present. These were in close agreement with those obtained later from the distillation curves of the diol acetates.

The methyl esters (20 c.c.) of the hydroxy-acids obtained by solvent distribution of total wool-wax fatty acids were separated further by distillation at 1 mm. in the micro-spinning-band fractionating column described in Part IV (*loc. cit.*) into the following main fractions: (1) b. p. 100—127° (0.8 c.c.); (2) b. p. 133.5—136.5° (1.0 c.c.); (3) b. p. 153.5—154.0° (9.8 c.c.); and (4) b. p. 165.5—172.5° (3.8 c.c.).

*Methyl 2-hydroxy-*n*-dodecanoate.* Fraction 1 was purified by counter-current distribution between hexane and 85% ethanol (40 transfers). The middle fraction, recrystallised from ether at -10° to -20°, formed a microcrystalline powder, m. p. 24—25°, $[\alpha]_D -3.0^\circ$ (*c*, 9 in CHCl_3) (Found: C, 68.0; H, 11.1; active H, 0.43%; sap. equiv., 234. $C_{12}H_{24}O_2$ requires C, 67.8; H, 11.4; active H, 0.44%; equiv., 230). The ester (94.3 mg.) was reduced with lithium aluminium hydride, the diol produced (48.1 mg.) was oxidised with periodic acid (Karnovsky and Rapson, *J. Soc. Chem. Ind.*, 1946, 65, 138), and the formaldehyde precipitated as the dimedone compound (74.6 mg.), m. p. 184—185° [$C_{12}H_{26}O_2$ (48.1 mg.) requires 69.2 mg. of dimedone compound, m. p. 189°].

The ester (182 mg.) gave the acid (128 mg.) which, twice recrystallised from acetone at -30°, formed a white powder (see Table) (Found: C, 66.9; H, 11.3; active H, 0.90%; equiv., 220. $C_{12}H_{24}O_2$ requires C, 66.6; H, 11.2; active H, 0.93%; equiv., 216). The acid (20 mg.) was oxidised with lead tetra-acetate, and the acid produced recrystallised from pentane at -20° as white powder, m. p. 26.5—27.0° (Found: equiv., 188. Calc. for $C_{11}H_{22}O_2$: equiv., 186), not depressing the m. p. of undecanoic acid, m. p. 27—27.5°, prepared by hydrogenation of undec-10-enoic acid. The 1° discrepancy in m. p. indicates slight impurity.

*Methyl 2-hydroxy-*n*-tetradecanoate.* Fraction 2 (1 c.c.) was purified by counter-current distribution and crystallised from ether and then acetone as a white powder, $[\alpha]_D -3.6^\circ$ (*c*, 9 in CHCl_3) (Found: C, 70.1; H, 12.0; active H, 0.39%; sap. equiv., 261. Calc. for $C_{14}H_{28}O_2$: C, 69.7; H, 11.7; active H, 0.39%; sap. equiv., 258).

The derived free acid crystallised from acetone at -30° as a white powder (see Table) (Found: C, 68.8; H, 11.5; active H, 0.89%; equiv., 243. Calc. for $C_{14}H_{28}O_2$: C, 68.8; H, 11.6; active H, 0.82%; equiv., 244).

Oxidation of the acid with lead tetra-acetate yielded *n*-tridecanoic acid, m. p. 40.5—41.0° (Found: equiv., 215. Calc. for $C_{13}H_{26}O_2$: equiv., 214), alone or mixed with an authentic sample, m. p. 41.0—41.5°, prepared by oxidation of synthetic (\pm)-2-hydroxy-*n*-tetradecanoic acid.

Methyl 2-hydroxyoctadecanoate isomers. Fraction 4 was purified by counter-current distribution and crystallised from ether and then acetone as a white powder, m. p. 38.5—39.5°, $[\alpha]_D -4.5^\circ$ (*c*, 6 in CHCl_3) (Found: C, 72.3; H, 12.2%; active H, 0.32%; sap. equiv., 316. Calc. for $C_{18}H_{36}O_2$: C, 72.6; H, 12.2; active H, 0.32%; equiv., 315).

The derived mixed acids crystallised from acetone as a white powder (*cf.* Table) (Found: C, 71.8; H, 12.0%; active H, 0.64%; equiv., 302. Calc. for $C_{18}H_{36}O_2$: C, 72.0; H, 12.1; active H, 0.64%; equiv., 300). Oxidation with lead tetra-acetate yielded an acid, m. p. 57—58° (Found: C, 75.5; H, 12.8%; equiv., 268. Calc. for $C_{17}H_{34}O_2$: C, 75.5; H, 12.7%; equiv., 270). The mixed m. p. with synthetic 15-methylhexadecanoic acid, m. p. 60.3—60.5°, was about 58—59°.

Synthesis of 15-Methylhexadecanoic Acid.—12-Methyltridecanoic acid (4.56 g.) (Part III, *J.*, 1953, 101) and ethyl hydrogen glutarate (9.4 g.; Hesse and Bücking, *Annalen*, 1949, 563, 31) were added to a solution of sodium (0.003 g.) in methanol (250 c.c.), and the mixture electrolysed to yield 15-methylhexadecanoic acid (1.88 g.). It was purified by counter-current distribution (*see* Part III). It crystallised from acetone in plates, m. p. 60.3—60.5° (Weitkamp, *loc. cit.*, records m. p. 60.2°) (Found: C, 75.5; H, 12.7%; equiv., 270. Calc. for $C_{17}H_{34}O_2$: C, 75.5; H, 12.7%; equiv., 270).

Synthesis of (\pm)- α -Hydroxy-acids.—In the synthesis of (\pm)- α -hydroxy-acids by bromination

of a fatty acid and subsequent hydrolysis, Bagard's method (*Bull. Soc. chim.*, 1907, 1, 30) which uses 0.33 mol. of phosphorus trichloride, gave a purer bromo-acid than did that of Clark and Taylor (*Org. Synth.*, 1941, Coll. Vol. I, p. 115).

Characterisation of the Hydroxy-acid Mixture by Fractionation of the Diols.—Wool-wax acids (300 g.), after methylation, were reduced with lithium aluminium hydride in the usual way, to yield a mixture of monohydric and dihydric alcohols (258 g.). Acetylation yielded the corresponding acetates which were distilled at 1 mm. into two main fractions: (a) b. p. up to 158° and (b) b. p. 158—205°. These were separately saponified and chromatographed on alumina (cf. Part IV). The two glycol fractions obtained were combined (ca. 56 g., 80%), acetylated, and distilled at 1 mm. in the micro-spinning-band fractionating column to yield main fractions: (1) b. p. 154.5°; (2) b. p. 172°; (3) b. p. 185°; and (4) b. p. 187—192°.

n-Tetradecane-1 : 2-diol. Fraction 1 was hydrolysed and the *diol* produced crystallised from hexane, and then methanol, as plates (Found: C, 73.0; H, 13.1. $C_{14}H_{30}O_2$ requires C, 73.0; H, 13.1%).

n-Hexadecane-1 : 2-diol. Fraction 2, the *diacetate*, crystallised from methanol as long needles, f. p. 18.4°, n_D^{20} 1.4394 (Found: C, 70.1; H, 11.3; Ac, 25.0. $C_{20}H_{38}O_4$ requires C, 70.1; H, 11.2; Ac, 25.1%), and gave the diol, plates (from hexane and then acetone) (Found: C, 74.5; H, 13.4. Calc. for $C_{16}H_{34}O_2$: C, 74.4; H, 13.3%).

16-Methylheptadecane-1 : 2-diol. Fraction 3 was hydrolysed to the diol, plates (from hexane) (Found: C, 75.6; H, 13.5. Calc. for $C_{18}H_{38}O_2$: C, 75.5; H, 13.4%). The pure diol was oxidised with lead tetra-acetate to 15-methylhexadecanoic acid, plates, m. p. 60.2° (Found: C, 75.4; H, 12.7%; equiv., 270. Calc. for $C_{17}H_{34}O_2$: C, 75.5; H, 12.7%; equiv., 270). The infra-red spectra of both the diol and the degraded acid confirmed the view that they contain a terminal isopropyl group.

n-Octadecane-1 : 2-diol. Fraction 4 was hydrolysed and crystallised from hexane, ethanol, carbon tetrachloride and methanol, to give the *diol* as plates (Found: C, 75.3; H, 13.5. $C_{18}H_{38}O_2$ requires C, 75.5; H, 13.4%). The infra-red absorption spectrum was identical with that of synthetic (\pm)-octadecane-1 : 2-diol (see Part IV).

For other physical constants see the Table.

The authors thank Dr. W. S. Rapson for many helpful discussions during the course of the work, Mr. F. W. G. Schöning for the microanalyses, Mr. C. A. J. Hoeve for the infra-red absorption spectra, and the South African Wool Board for a fellowship awarded to one of them (E. v. R.). This paper is published by permission of the South African Council for Scientific and Industrial Research.

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PRETORIA, SOUTH AFRICA.

[Received, August 4th, 1953.]