THE CONSTITUTION OF WOOL WAX

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THE earliest workers [1—4] showed that glycerol does not occur in wool wax, which is therefore a true wax, *i.e.*, a mixture of naturally occurring esters of alcohols other than glycerol. Elementary analysis shows the presence of traces of nitrogen and phosphorus,[4] but compounds containing these elements have not been concentrated. The nitrogenous material is thought to be contained in the yellow colouring matter, lanaurin, which is related to bilirubin.[5] Sulphur derived from the sheep-dip and from the wool itself, is a common impurity in crude samples.

Until recently the unique complexity of wool wax has defied analysis, and it has been quite common for one set of workers to be unable to isolate a component reported by others. Few attempts, all of them unsuccessful, have been made to separate and identify individual esters. The usual method of investigation is to hydrolyse the wax and examine the resultant acids and alcohols separately; in this way about 60% of the acids and a similar percentage of the alcohols have been identified. The acids and alcohols occur in approximately equal amounts, each group constituting about 50% of the wax.

Acids

Until quite recently, there was no evidence of the natural occurrence of any saturated higher fatty acids other than those of the normal series, and the acids obtained from wool wax were assumed to belong to this group. The fact that the physical constants did not correspond was attributed to the presence of impurities, but as most workers were unable to obtain any acid in a reasonable state of purity, and also because derivatives were seldom made, the identification being based solely on the melting point, elementary analysis, and equivalent weight, much confusion resulted. For example, the presence of myristic,[6, 7] palmitic,[4, 8] and stearic acids [4, 8] has been reported by some authors and denied by others (myristic,[9] palmitic,[7] stearic [7]). Similarly, cerotic acid obtained from beeswax was said to differ from,[10] and alternatively be identical with, that from wool wax.[12, 11]

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[1] Chevreul, *Compt. rend.*, 1856, **42,** 130.

[2] Hartmann, " Über den Fettschweiss der Schafwolle ", Göttingen, 1868.

[3] Schulze, *Ber.*, 1872, **5,** 1075; 1873, **6,** 251.

[4] Drummond and Baker, *J. Soc. Chem. Ind.*, 1929, **48,** 232T.

[5] Rimington and Stewart, *Proc. Roy. Soc.*, 1932, *B*, **110,** 75.

[6] Darmstädter and Lifschütz, *Ber.*, 1896, **29,** 618, 1474.

[7] *Idem, ibid.*, p. 2890.

[8] de Sanctis, *Gazzetta*, 1894, **24,** 14.

[9] Röhmann, *Biochem. Z.*, 1916, **77,** 298.

[10] Darmstädter and Lifschütz, *Ber.*, 1898, **31,** 97, 1122.

[11] Grassow, *Biochem. Z.*, 1924, **148,** 61.

[12] Heiduschka and Nier, *J. pr. Chem.*, 1937, **149,** 98.

Nevertheless, from the behaviour of the mixed products, the presence of a series of saturated and a series of hydroxy-acids was deduced. The chief method of separation was the tedious one of fractional crystallisation of the acids themselves or their lead or magnesium salts. Later, low-pressure distillation of the methyl esters was also employed. By using a combination of these methods, all workers have been able to obtain one of two major fractions with median melting points at 72° and 77° and compositions corresponding approximately to $C_{24}H_{48}O_2$ and $C_{26}H_{52}O_2$. These are normally referred to as carnaubic [6, 7] and cerotic [4, 8, 9, 11, 13, 14] acids, respectively. These acids, if regarded as single substances, which many authors deny, are certainly not of the *n*-series [14, 15] or of the *iso*-series (terminal *iso*propyl residue). Mixed melting points of members of the *n*- and *iso*-series are depressed by 10—15°.

$C_{24}H_{48}O_2$.	M.p.	$C_{26}H_{52}O_2$.	M.p.
Carnaubic acid	73°	Cerotic acid	78°
n-Tetracosanoic acid	84	*n*-Hexacosanoic acid	88
*iso*Tetracosanoic acid. . . . . (22-methyltricosanoic acid)	83	*iso*Hexacosanoic acid . . . . . (24-methylpentacosanoic acid)	87

Darmstädter and Lifschütz [6, 7] reported the isolation of two hydroxy-acids, " lanoceric " acid, $C_{30}H_{58}O_4$, m.p. 103°, which gave " lanoceric lactone ", m.p. 87°, when warmed with acids, and " lanopalmic " acid, $C_{16}H_{32}O_2$. Further evidence for the presence of hydroxy-acids was obtained by Lewkowitsch,[16] Nozoe,[17] Bertram,[18] and by Röhmann,[9] who found lanoceric and hydroxystearic acids but not hydroxypalmitic. The presence of a series of weakly dextrorotatory, saturated acids was also indicated but none was identified. In later work by Drummond and Baker,[4] however, neither hydroxy-acids nor optically active compounds could be found.

" Lanopalmic " acid was shown by Kuwata [19] to be α-hydroxypalmitic acid by oxidation of the methyl ester to α-ketopalmitate and then to *n*-pentadecanoic acid, both compounds being identical with the known ones. The constitution of " lanoceric" acid still remains obscure. It has been isolated by Grassow,[11] Heiduschka and Nier,[12] Kuwata and Ishii,[15] and more recently by Lipson.[20] Grassow was unable to acetylate the acid and therefore doubts the existence of a free hydroxyl group ; at the same time neither this nor the derivative " lanoceric lactone " can be a lactone because they consume alkali only after prolonged boiling with alcoholic potash.

The first suggestion that the saturated wool-wax acids are substantially of unknown structure and not of the *n*-series came from Abraham and Hilditch [14] in 1935. This conclusion was based on the observation that the melting points of the acids of a given molecular weight, determined by

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[13] Schulze and Urich, *Ber.*, 1874, **7**, 570 ; *J. pr. Chem.*, 1874, **9**, 321.

[14] Abraham and Hilditch, *J. Soc. Chem. Ind.*, 1935, **54**, 398.

[15] Kuwata and Ishii, *J. Soc. Chem. Ind. Japan* (Suppl.), 1936, **39**, *B*, 227.

[16] *J. Soc. Chem. Ind.*, 1892, **11**, 134 ; 1896, **15**, 14.

[17] Jap. Pat. 153,628 (*Chem. Abs.*, 1949, **43**, 3219).

[18] *J. Amer. Oil Chem. Soc.*, 1949, **26**, 454.

[19] *J. Amer. Chem. Soc.*, 1938, **60**, 559.

[20] *J. Coun. Sci. Ind. Res.* (*Australia*), 1940, **13**, 273.

Rast's method and by neutralisation, were far too low. Cerotic acid was isolated from several samples of wax, and the consensus of analytical figures obtained by these and other authors allows variation from $C_{25}H_{50}O_2$ to $C_{27}H_{54}O_2$, the latter structure being slightly more favoured than the others. As many samples had a weak dextrorotation, it was suggested that wool-wax acids were built up in a similar way to the terpenes, containing multiples of five carbon atoms. Further evidence for the presence of hitherto unknown acids was provided by Kuwata and Ishii,[15] who isolated saturated compounds containing 14, 16, 18, and 20 carbon atoms for which the generic term " lano-" acids was proposed. These acids and their amides all gave depressed melting points when mixed with corresponding members of the *n*-series.

In 1945 Weitkamp [21] identified no less than 32 acids, and in retrospect, it is easy to see why earlier workers were unable to obtain pure products. The methyl esters were fractionated in a column with a height equivalent to 100 theoretical plates, the extended distillation technique being used. The acids isolated fell into four series. For two groups of hitherto unknown acids he deduced that they were homologous from the crystal habit, the melting-point sequence, neutralisation equivalent, and, where possible, by conversion of one member into the next homologue. Having established that the series were homologous, it was necessary to identify only one member in order to place all the others. The four groups found are hydroxy-, *n*-, *iso*- (terminal *iso*propyl group), and *anteiso*-acids (methyl group on the antepenultimate carbon atom).

In the two branched-chain series the position of the carbon atom at which the chain branches was discovered by a new method of analysis. The fact that the solidification-point curve for a binary mixture of adjacent, even-numbered fatty acids shows two breaks is explained by assuming the formation of an intermolecular compound. If one of the components has a side group it interferes with compound formation and the solidification curve exhibits only one break. If, however, the length of the *n*-acid is less than the straight portion of the branched acid, the normal two-break curve is observed. Hence, by progressively changing the length of the admixed *n*-acid, the position of the tertiary carbon can be found.

Hydroxy-acids.—About 3½% of the total acids were found to be of this type. Only two members were isolated, hydroxymyristic and hydroxypalmitic, and as reported by Kuwata these are weakly lævorotatory :

	α-Hydroxypalmitic acid.			
	Weitkamp.	Kuwata.	Synthetic.	Other authors.
M.p. of acid	93·6°	86—87°	(±)86°	87° [7, 14]
M.p. of methyl ester	45·6	45—46	58	
M.p. of keto-methyl ester . . .		47	47	

Normal Acids.—These make up about 7% of the total. The nine even-numbered carbon chains from 10 to 26 were found, all but myristic and palmitic being minor components. No depression of melting point was

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[21] *J. Amer. Chem. Soc.*, 1945, **67**, 447.

observed when each acid was mixed with an authentic sample of the *n*-series.

iso-**Acids.**—These account for about 22% of the total. Ten even-numbered carbon chains are present from 10 to 28, the first two and the last being minor constituents. Although these acids have a terminal *iso*propyl group, their melting points are very near to the corresponding ones of the *n*-series, but they are easily distinguished from the latter by means of mixed melting points, which give a 10—15° depression. By carrying out the nitrile synthesis twice on *iso*palmitic acid, a product identical with *iso*stearic acid was obtained. A slight discrepancy in the melting points of wool wax *iso*myristic acid and the synthetic one [22] has been eliminated by the resynthesis of the acid.[23] *iso*Stearic acid was converted into *iso*nonadecanoic acid by the nitrile synthesis and the product was identical with the compound made by Cason : [24]

*iso*Nonadecanoic acid derivatives :	Synthetic.	Wool wax.
M.p. of acid	67·5°	66·9°
M.p. of amide	100—101	101·2
M.p. of tribromoanilide . . .	112·3	112·1

anteiso-**Acids.**—These constitute about 30% of the total. All *odd*-numbered carbon chains from 9 to 31 except 29 were found, the seven members with 15 to 27 carbon atoms being the most abundant. Thermal analysis showed that branching of the chain took place at the fourth atom from the end. Two end-groups were therefore possible, *sec.*-butyl and *tert.*-butyl. The second possibility was eliminated when the acids were found to be optically active. At the time there were no data for such optically active acids, but since then the method of structure analysis has been fully vindicated by the synthesis of (+)-6-methyloctanoic,[25] (+)-14-methylpalmitic,[26] and other acids.[27] *

	14-Methylpalmitic acid.		
	Synthetic.	Wool wax.	Mixed.
M.p. of acid	36—37°	36·8°	37°
M.p. of amide	88—89	90·9	90
$[\alpha]_D$ of acid	+ 5·2	+ 5·2	

Although the two series of branched-chain amides do not give smooth curves when melting points are plotted against carbon content, the long *X*-ray spacings [28] show that the series are indeed homologous. The curves show a four-atom periodicity which is explained by assuming that the molecular structure is spiral rather than zig-zag.

It is interesting to note that, so far, no unsaturated acid has been properly identified although the iodine value of the mixed acids indicates that there

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[22] Fordyce and Johnson, *J. Amer. Chem. Soc.*, 1933, **55**, 3368.

[23] Rapson, *Chem. and Ind.*, 1951, 366.

[24] *J. Amer. Chem. Soc.*, 1942, **64**, 1106.

[25] Crombie and Harper, *J.*, 1950, 2685.

[26] English and Vellick, *J. Biol. Chem.*, 1946, **160**, 473.

[27] Nunn, *J.*, in the press.

[28] Vellick, *J. Amer. Chem. Soc.*, 1947, **69**, 2317.

* Long-chain acids are numbered according to the Geneva convention ($CO_2H = 1$) throughout.

should be about 15% of such acids. It is difficult to see why Weitkamp did not succeed in isolating any of them. In the preparation of the mixed methyl esters for distillation, 12% was lost during the decolorising treatment with Attapulgus clay. It must therefore be assumed that all the unsaturated esters were removed in this process.

The occurrence of branched-chain fatty acids has been found to be more common than was formerly believed.[29—31] The tubercle bacillus has yielded tuberculostearic (10-methylstearic), phthioic (3 : 13 : 19-trimethyltricosanoic), and other (unidentified) trimethyl-substituted lævo- and dextro-rotatory acids containing between 24 and 30 carbon atoms. (+)-6-Methyloctanoic acid has been isolated from the hydrolytic degradation products of the polymyxins,[32] a series of related antibiotics from *B. Polymyxa,* and phytomonic acid (10- or 11-methylnonadecanoic) from *Phytomonas tumefaciens.*[33] If the identity of wool-wax cerotic acid with beeswax cerotic acid is admitted,[11, 12] then it is likely that branched-chain acids will also be found in beeswax.

The configuration of the *anteiso*-acids is identical with that of D(—)-2-methylbutanol and also with L(—)-*iso*leucine. This relationship suggests that biosynthesis may begin from *iso*leucine. Similarly, the *iso*-acids may start from valine.[26]

Alcohols

In 1868 Hartmann [2] showed that wool wax did not contain glycerol. He found cholesterol, which was identified by its physical properties although its structure was then unknown. Four years later, Schulze [3] found another alcohol of approximately the same molecular weight which was dextrorotatory. This he named *iso*cholesterol, and in order to satisfy the alcohol equivalent of the acids liberated by hydrolysis of the esters, he inferred the presence of alcohols of lower molecular weight which were assumed to be of the aliphatic series. At present only these three groups of alcohols are known to occur in wool wax, namely, sterols, *iso*cholesterol, and aliphatic alcohols.

Aliphatic Alcohols.—After the removal of cholesterol and *iso*cholesterol from the mixed alcohols an optically inactive residue remains from which nearly all workers have isolated ceryl alcohol, a monohydric substance, $C_{26}H_{53}OH$. As in the case of the acids, however, the melting points of this and related alcohols are too low to allow them to be identified as members of the *n*-series. This view is confirmed by oxidation of the alcohol to the corresponding acid. Matthes and Sauder's evidence [34] indicates that these alcohols may be members of the normal series, for it was shown that " carnaubyl " alcohol, m.p. 68°, obtained from laurel oil, was a mixture of an alcohol melting at 88° and a hydrocarbon melting at 69°. For wool

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[29] Brownlee, *Ann. Reports,* 1948, **45,** 292. [30] Bowman, *ibid.,* 1949, **46,** 160.

[31] Weitzel, *Angew. Chem.,* 1948, **60,** *A,* 263.

[32] Catch, Jones, and Wilkinson, *Biochem. J.,* 1948, **43,** xxvii.

[33] Vellick, *J. Biol. Chem.,* 1944, **152,** 533 ; **156,** 101.

[34] *Arch. Pharm.,* 1908, **246,** 169.

wax, however, the hydrocarbon content is estimated at less than 1%,[35] so the possibility of compounds analogous to the *iso*- and *anteiso*-acids cannot be excluded.

Buisine [36] states that ceryl alcohol is a major component, and Lewkowitsch,[16] Darmstädter and Lifschütz,[7] Röhmann,[9] Heiduschka and Nier,[12] and Lederer *et al.*[35] concur. The physical constants obtained by these workers are:

	L.	D. & L.	R.	H. & N.	L. *et al.*	*n*-Hexacosanol.
M.p. of alcohol	57—58°	78°	77°	75·5°	73—76°	80°
M.p. of acetate	37—39		60		56—61	65
M.p. of corresponding acid.		69—70		77·5		88

Many of these samples showed changes in melting points on further recrystallisation, so the identity of ceryl alcohol as a single compound cannot seriously be considered.

After removal of cholesterol by precipitation with digitonin, and of *iso*cholesterol by recrystallisation from methanol, the residue was subjected to low-pressure fractional distillation. Among the distillates, Drummond and Baker [4] were able to identify cetyl alcohol:

	Cetyl alcohol.	Found (D. & B.).
M.p. of alcohol	51°	50—50·5°
M.p. of phenylurethane . . .	73—74	72

Two hitherto unknown alcohols have been reported by Kuwata and Katuno.[37] One is an isomer of octadecanol named "lano-octadecanol", and the other is a diol containing an *odd* number of carbon atoms, "lanyl" alcohol, $C_{21}H_{42}(OH)_2$.

A mixture of monohydric alcohols and diols has been separated from the mixed alcohols by treatment with urea.[38, 39] Separation of one group from the other can be achieved by adsorption on an alumina column [38] or by further treatment with urea.[39] Both groups are mixtures of several members of homologous series, the constitution of which is likely to be worked out in the near future. The diols are optically active. The monohydric group contains members of the *n*-series, *n*-docosanol having been identified by oxidation to *n*-docosanoic acid.[39]

Sterols.—Cholesterol is the only alcoholic constituent that has been found by all workers. The physical properties of the earlier preparations showed some variation, indicating the presence of closely related impurities, but it was not until 1930 that Schönheimer [40] demonstrated that the dihydro-derivative almost invariably accompanies cholesterol in Nature. The unsaturated cholesterol is first precipitated as the dibromide from an alcoholic solution, and the dihydro-derivative is then precipitated with digitonin. It was found that, of the total amount of material that could be precipitated

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[35] Daniel, Lederer, and Velluz, *Bull. Soc. Chim. biol.*, 1945, **27**, 218.

[36] *Bull. Soc. chim.*, 1887, **72**, 201.

[37] *J. Soc. Chem. Ind. Japan* (Suppl.), 1938, **41**, *B*, 227.

[38] Von Rudloff, *Chem. and Ind.*, 1951, 338.

[39] Tiedt and Truter, unpublished.

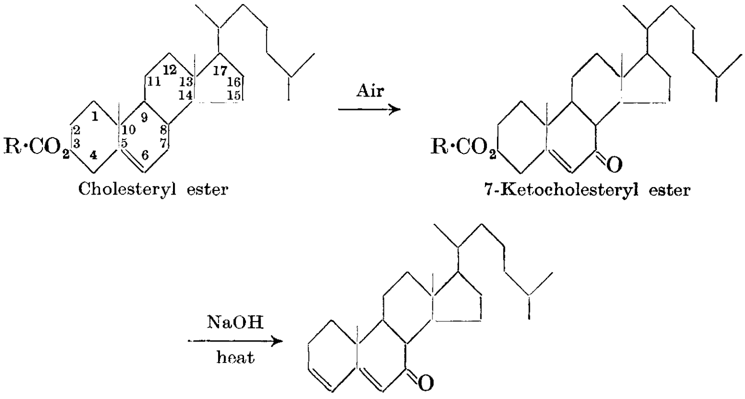
[40] *Z. physiol. Chem.*, 1930, **192**, 86.

by digitonin, 14—19% was fully saturated.[41] Later, it was reported that the sterol fraction from wool wax gave a strong Lifschütz reaction, thus indicating the presence of cholesterol oxide.[42] It is thought that this is not a natural product but arises from aerial oxidation.

The presence of at least two ketones has been demonstrated by chromatographic analysis on an alumina column.[35] One is still unidentified but is thought to be a mixture of cholesta-4 : 6-dien-3-one and cholest-4-en-3-one. The other is cholesta-3 : 5-dien-7-one which is notable for its strong lævorotation :

	Cholesta-3 : 5-dien-7-one.	Found (D., L. & V.).
M.p. of ketone	112—114°	112—113°
$[\alpha]_D$ (in benzene)	− 303 ± 5°	− 305 ± 5°

This ketone is not a natural product but is formed during hot saponification thus :



Cholesteryl ester

7-Ketocholesteryl ester

Cholesta-3 : 5-dien-7-one

In fact, this reaction is the basis of Mauthner and Suida's synthesis of cholesta-3 : 5-dien-7-one. At present it is not known whether the 7-ketocholesteryl ester is a natural product, because colloidal cholesterol can be oxidised in air to 7-hydroxy- and then to 7-keto-cholesterol.[43] It may well be that aerial oxidation is the reason for the occurrence of 7-ketocholesteryl esters in wool wax.

It has been claimed [44] that wool wax contains members of the vitamin D group or their precursors, but both ultra-violet absorption studies and biological experiments have shown that this is not so.[4]

*iso***Cholesterol.**—While trying to separate cholesterol from wool-wax alcohols Schulze[3] noticed that it was usually accompanied by a gelatinous or flocculent precipitate. By slow evaporation of an ethereal solution he was able to separate platelets of cholesteryl benzoate from needles of the other benzoate. The parent alcohol had roughly the same elementary composition as cholesterol but it had a different crystal habit and a slightly lower

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[41] Behring, Hummel, and Schönheimer, *Z. physiol. Chem.*, 1930, **192,** 93.

[42] Behring and Schönheimer, *ibid.*, p. 111.

[43] Bergström and Winterstein, *J. Biol. Chem.*, 1941, **137,** 785.

[44] Hess, Weinstock, and Helman, *ibid.*, 1925, **63,** 305.

melting point and it was dextrorotatory. Schulze named it "*iso*cholesterol".[3] The existence of this alcohol was questioned by several workers, so Schulze [45] repeated the experiments and admitted that *iso*cholesterol might be a mixture of alcohols because repeated recrystallisation of the benzoate gradually raised the melting point. Several workers [46–48] were able to confirm these results and Cohen [49] discovered what was apparently the same substance in the latex of trees of the *Euphorbia* species.

In 1930 Windaus and Tschesche [50] showed that *iso*cholesterol is a mixture of two alcohols. They found 8% of what they called "agnosterol" and the remainder was called "lanosterol" (after Drummond and Baker). It was shown that the Liebermann–Burchardt reaction with *iso*cholesterol differed from that of all known sterols: whereas cholesterol gave a green colour and no fluorescence, *iso*cholesterol gives a yellow-brown colour and an intense green fluorescence. The difference had been noticed before,[46] and has recently been developed into a quantitative test by Lederer and Tchen.[51] The determination must be carried out under standard conditions and is accurate to 0·5%. It is based on the fact that the *iso*cholesterol solution shows a sharp absorption peak at 458 mμ and does not interfere with the determination of cholesterol. The cholesterol solution, however, has a broad band with the absorption maximum at 620 mμ, and allowance has to be made for cholesterol when determining *iso*cholesterol. The method does not distinguish between the different constituents of *iso*cholesterol. It is by no means certain that agnosterol is a constant component of wool wax, because only minute traces have been found in all laboratory-produced waxes. It may be derived from lanosterol during commercial processing, although if it is, the mechanism is not similar to that by which cholesta-3 : 5-dien-7-one is produced, because there is no increase in the amount of agnosterol if *iso*cholesterol is boiled with alkali. [52]

Fractional crystallisation and chromatographic analysis of acetylated *iso*cholesterol have led to the separation of the dihydro-derivatives of both lanosterol and agnosterol.[65, 66] Only a trace of agnosterol is present but the dihydro-derivative makes up about 20% of the total, the remainder being equal quantities of lanosterol and dihydrolanosterol. Only these four members of the *iso*cholesterol group have been identified.

Structure of Lanosterol.—The complete structure of the *iso*cholesterol alcohols is still unknown, but the researches of Dorée,[52–60] McGhie,[57–64]

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[45] *Ber.*, 1898, **31**, 1200.

[46] Darmstädter and Lifschütz, *ibid.*, p. 97.

[47] Ruppel, *Z. physiol. Chem.*, 1895, **21**, 122.

[48] Moreschi, *Atti R. Accad. Lincei*, 1910, **19**, 53.

[49] *Arch. Pharm.*, 1908, **246**, 518, 592.

[50] *Z. physiol. Chem.*, 1930, **190**, 51.

[51] *Bull. Soc. Chim. biol.*, 1945, **27**, 419.

[52] Dorée and Garratt, *J. Soc. Chem. Ind.*, 1933, **52**, 141.

[53] *Idem, ibid.*, p. 335T.

[54] Dorée and Petrow, *J.*, 1936, 1562.

[55] Dorée and Bellamy, *J.*, 1941, 172.

[56] *Idem, ibid.*, p. 176.

[57] Dorée, McGhie, and Kurzer, *J.*, 1947, 1467.

[58] *Idem, J.*, 1948, 988.

[59] *Idem, J.*, 1949, 570.

[60] *Idem, J.*, 1949, S 167.

[61] Birchenough and McGhie, *J.*, 1949, 2038.

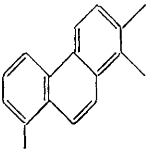
[62] *Idem, J.*, 1950, 1249.

[63] Cavalla and McGhie, *J.*, 1951, 744.

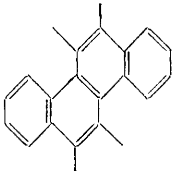
[64] *Idem, J.*, 1951, 834.

Ruzicka[65–69] and others have thrown considerable light on their interrelations. It is established that lanosterol has the composition $C_{30}H_{50}O$, and contains two double bonds one of which is inert, *i.e.*, cannot be hydrogenated.[50, 53, 65, 70, 71, 72] Agnosterol, $C_{30}H_{48}O$, contains three double bonds two of which are inert. Both alcohols, therefore, have a tetracyclic carbon skeleton. They are not true sterols[50, 53, 74, 73] and it has been suggested that they are members of the triterpene series. The close resemblance to substances obtained from raw rubber,[49] and the fact that the only dehydrogenation product that has been identified is also obtained from other triterpenes, supports this view.

Dehydrogenation experiments have not led to any definite conclusion with respect to the tetracyclic skeleton. From the reaction with selenium, 1 : 2 : 8-trimethylphenanthrene and an unidentified, higher-molecular-weight hydrocarbon have been obtained, and the failure of platinum dehydrogenation indicated that all the rings contain at least one quaternary carbon atom.[73] Ruzicka *et al.*[65] confirm that 1 : 2 : 8-trimethylphenanthrene is the main product of selenium dehydrogenation. Another product has an ultra-violet spectrogram very similar to, but not identical with, that of 1 : 2 : 7 : 8-tetramethylchrysene.



1 : 2 : 8-Trimethylphenanthrene



1 : 2 : 7 : 8-Tetramethylchrysene

Treatment of lanosteryl acetate with ozone gives acetone, which can be isolated in 35—40% yield, and an acid, $C_{27}H_{44}O_3$, containing one double bond can be obtained from the mother-liquor. The ultra-violet spectrogram excludes the possibility of this being an $\alpha\beta$-unsaturated acid.[65] Lanosterol must therefore contain a side chain of at least four carbon atoms which ends in an *iso*propylidene group. It is this group that contains the easily hydrogenated double bond.

Oxidation of dihydrolanosteryl acetate (I) gives a ketone for which the ultra-violet and infra-red spectra indicate $\alpha\beta$-unsaturation, the double bond lying between two quaternary carbon atoms (III),[65] and a yellow, unsaturated 1 : 4-diketone (V). Hence, unless the double bond has migrated, it is adjacent to two methylene groups. The inert double bond in dihydro-

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[65] Ruzicka, Rey, and Muhr, *Helv. Chim. Acta,* 1944, **27,** 472.

[66] Ruzicka, Denss, and Jeger, *ibid.*, 1945, **28,** 759.

[67] *Idem, ibid.*, 1946, **29,** 204.

[68] *Idem, ibid.*, 1948, **31,** 818.

[69] Vosser, Montavon, Günthard, Jeger, and Ruzicka, *ibid.*, 1950, **33,** 1893.

[70] Marker, Wittle, and Mixon, *J. Amer. Chem. Soc.*, 1937, **59,** 1368, 2289.

[71] Windaus, von Werder, and Geschaider, *Ber.*, 1932, **65,** 1006.

[72] Wieland, Passendach, and Ballauf, *Annalen,* 1937, **529,** 68.

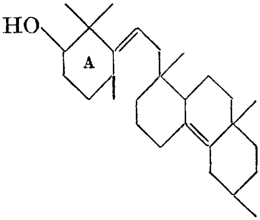
[73] Schulze, *Z. physiol. Chem.*, 1936, **238,** 35.

lanosteryl acetate is not attacked by ozone, but the adjacent methylene groups can be oxidised to give the same products as are obtained from chromic acid oxidation. After hydrolysis of the yellow dihydrodiketolanosteryl acetate (V) and oxidation of the resultant alcohol to the ketone, the product has an absorption spectrum very similar to that of the parent alcohol. Hence the hydroxyl group is in an isolated position with respect to the 1 : 4-unsaturated dione system. A minor product of vigorous oxidation of dihydrolanosteryl acetate is an orange triketone (IX), in which the third keto-group is conjugated with the unsaturated 1 : 4-dione system.

Dihydroagnosterol is an isomer of lanosterol (γ-lanosterol, II). Before its isolation from wool wax [65] it had been prepared from lanosterol by Marker *et al.*[70] It is obtained by dehydrogenation of lanosterol with *N*-bromosuccinimide [60] or with perbenzoic acid,[62] simultaneous migration of the original double bond taking place.[69] Oxidation of dihydroagnosteryl acetate gives dihydroketolanosteryl acetate (III), didehydrodihydroketolanosteryl acetate (IV) and finally dehydrodihydrodiketolanosteryl acetate (VI).

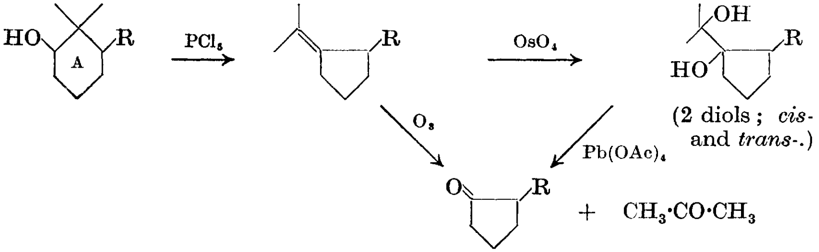
As (I) can be oxidised to (V) in three different ways—with chromic acid,[62, 65] with ozone, or with hydrogen peroxide [69]—it is assumed that bond migration has not taken place. Oxidation of (V) with selenium dioxide gives (VI) or dehydrodihydrotriketolanosteryl acetate (VII), according to conditions. The double bond introduced in this way is conjugated with the 1 : 4-dione system,[69] and the triketone contains the α-diketo-group. Although the α-diketone does not give the characteristic reactions with *o*-phenylenediamine or ferric chloride, its presence has, nevertheless, been demonstrated by oxidation with alkaline peroxide. The product is a tricyclic dicarboxylic acid (VIII) containing two double bonds and a hydroxy- and a keto-group.[63]

Both the diketo- and the triketo-compound are reduced by zinc and acetic acid to colourless diketolanostanyl acetate (X).[58] Although this compound contains two keto-groups it forms only mono-derivatives, *e.g.*, a monoxime. After desulphurisation of the ethylene thioacetal (XI) with Raney nickel and reduction of the remaining keto-group, it is found that the resultant alcohol cannot be acetylated. Dehydration of the alcohol (XII) gives an isomer of dihydrolanosteryl acetate (XIII) which can be catalytically hydrogenated to tetrahydrolanosteryl acetate, from which, after hydrolysis and reduction, the parent hydrocarbon of the series, lanostane (XIV), is obtained.



(XV.)

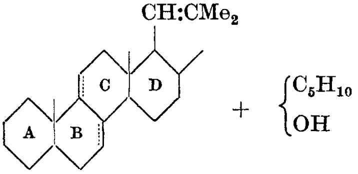
Doree and Bellamy [56] provisionally formulate lanosterol as (XV). The chief objections to this formula are that acetone is not a likely product from ozonolysis, and 1 : 2 : 8-trimethylphenanthrene is an unlikely dehydrogenation product. The structure of ring A has been elucidated by the following series of reactions,[60, 68] both lanosterol and agnosterol behaving in the same way :



(2 diols ; *cis*- and *trans*-.)

(one ketone + acetone)

Wieland *et al.*[72] noticed the resemblance between kryptosterol from yeast and lanosterol and were able to show that out of a large number of derivatives only two had appreciably different physical constants.[74] They were led to suppose that kryptosterol and lanosterol were isomers with

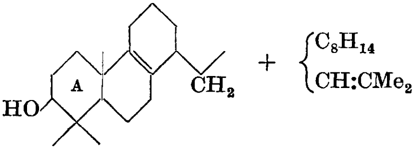


(XVI.)

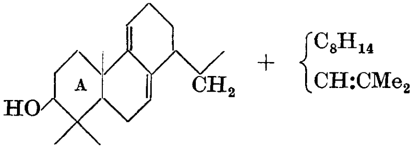
respect to the location of the passive double bond, the partial formulation[75] (XVI) being preferred, in which the dotted lines indicate the positions of the double bonds that give rise to the kryptosterol–lanosterol isomerism.

Ruzicka *et al.*[66] have shown that lanosterol is, in fact, identical with kryptosterol, Wieland's error being caused by the fact that lanosterol and dihydrolanosterol form mixed crystals that are very difficult to separate. Wieland's sample was actually an equal mixture of lanosterol and the dihydro-derivative. Wieland *et al.* suggest that the hydroxyl group probably occurs in the proximity of the side chain, perhaps on the angular methyl group between rings C and D.

Ruzicka *et al.*[69] prefer the partial formulation



Lanosterol



Agnosterol

Earlier work [65] makes a slight modification in ring A desirable. Oxidation of hydroxymethylenelanosterone gives a diacid, the dimethyl ester of

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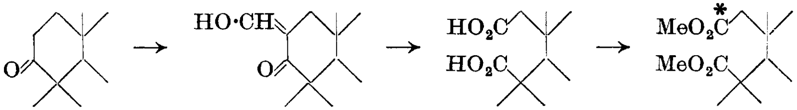
[74] Wieland and Joost, *Annalen*, 1941, **546**, 103.

[75] Wieland and Benend, *Z. physiol. Chem.*, 1942, **274**, 215.

which could not be hydrolysed even on prolonged boiling with N/2-alkali. Hence both carboxyl groups are probably attached to tertiary carbon atoms, but Ruzicka's scheme,[69] as written above, gives rise to a diester containing one easily hydrolysable group :



* Easily hydrolysable ester group.

Lanosterol is precipitated by digitonin, and because of the specificity of the reaction it can be inferred that its configuration is the same as that of cholesterol in the relevant respects : the hydroxyl must have the 3β-orientation, and as an acyloxy-group in the 4-position prevents precipitation,[76] a *gem*-dimethyl group may do so too. If the *gem*-dimethyl group is transferred to the 2-position then this difficulty, as well as the objection to the easily hydrolysed dimethyl ester (see above), is overcome without prejudice to the known behaviour on dehydration.

According to Wieland and Joost,[74] oxidation of lanosterol under vigorous conditions yields a keto-acid with the loss of five carbon atoms. Other workers have found acids which have different physical constants but all agree on the loss of five atoms.[55, 70] Hence it is probable that there is a side chain of six members which is terminated by an *iso*propylidene residue.

Of the eight undistributed carbon atoms in Ruzicka's formula, two are necessary for the side chain and three to complete the third ring, leaving three to be placed. As there are only two possible angular positions, it seems likely that the non-angular position is occupied by a *gem*-dimethyl group, leaving one angular methyl on $C_{(13)}$. The inert keto-group at $C_{(11)}$ resembles that of the sterol series.

Some of the foregoing relationships are illustrated in the scheme on p. 402.

Origin of iso*Cholesterol.*—With the development of a quantitative method of examination, it was found that *iso*cholesterol was absent from human hair grease and from that of cattle and rabbits, but present in that of the goat, camel, and llama. Hence it was concluded that it is a characteristic metabolic product of certain ruminants,[57] particularly the sheep. Later, investigation of the body fats from all the sheep's organs [77] showed that *iso*cholesterol occurred only in the skin, and that the proportions of cholesterol and *iso*cholesterol in it are approximately the same as in wool wax. A trace (less than 0·02%) has been detected in butter fat.[78] *iso*Cholesterol must, therefore, be synthesised by the skin.

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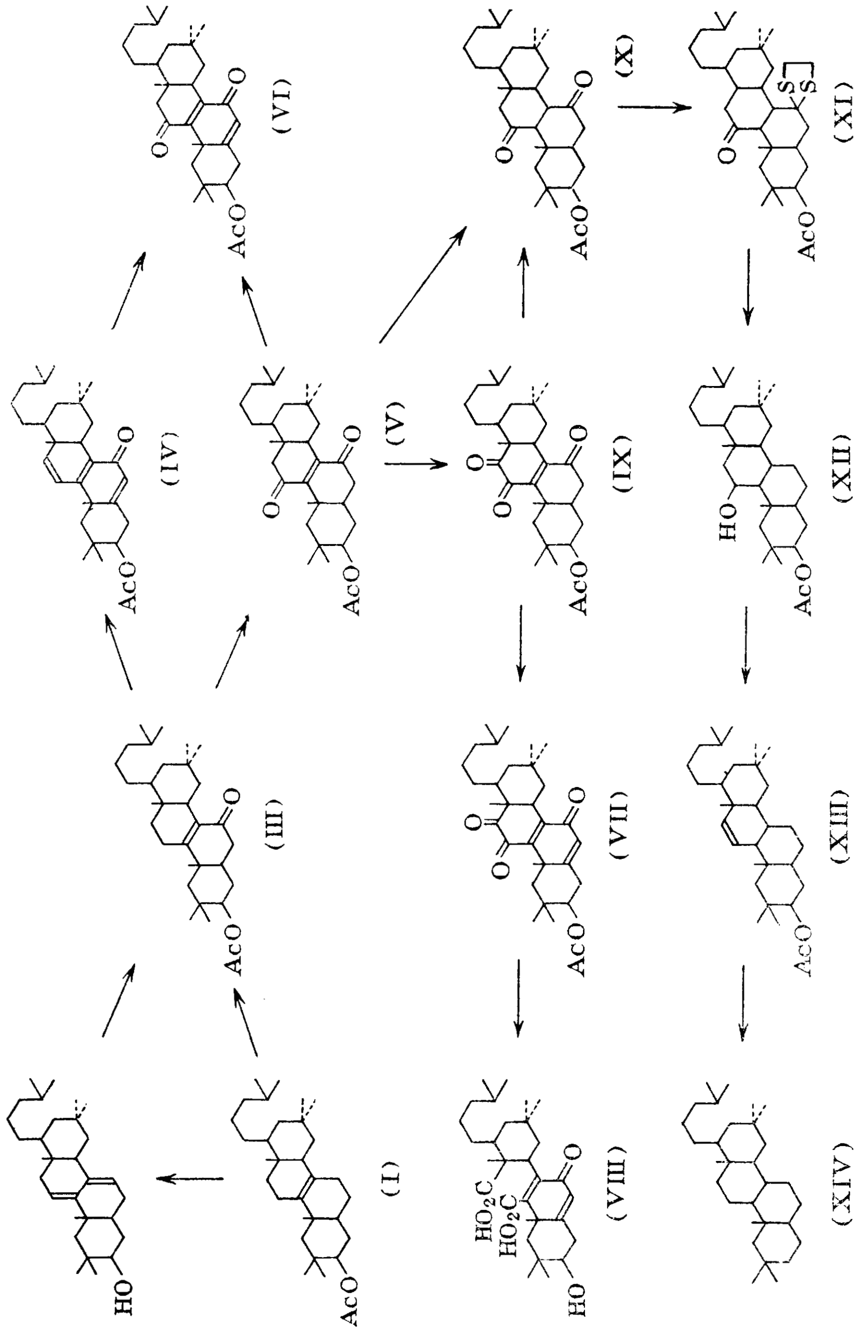
[76] Fieser and Fieser, " Natural Products related to Phenanthrene ", Reinhold, 3rd edtn., 1949, pp. 102, 173.

[77] Lederer and Mercier, *Biochim. Biophys. Acta,* 1948, **2,** 91.

[78] Morice, *J.*, 1951, 1200.


(I)
(III)
(IV)
(V)
(VI)
(VII)
(VIII)
(IX)
(X)
(XI)
(XII)
(XIII)
(XIV)
HO
AcO
O
HO_2C
HO_2C
S
S

Hydrocarbons

Traces of unidentified low-melting hydrocarbons have been found. It has been proved that these are part of the wax itself and not adventitious impurities.[35]

Esters

In spite of the number of acidic and alcoholic constituents that have been found, so far no single ester has been identified. It is usually assumed that the esters are formed by simple combination of the acids with the alcohols, but measurement of the molecular weight of wool wax (*ca.* 800) shows that it is much higher than that calculated on the basis of simple combination (*ca.* 600) or from the saponification equivalent (*ca.* 550). This is attributed to the presence of di-esters of hydroxy-acids, the hydroxyl value of the acids supporting such a view.[18] Further evidence to disprove the assumption of random combination is obtained from the behaviour of the wax towards urea. Wool-wax alcohols contain about 20% of straight-chain material,[38, 39] and the acids more than 60%. Random combination would result in the presence of 12% of straight-chain esters which could combine with urea. In fact, the amount of such material is negligible, and known straight-chain esters can be recovered quantitatively after admixture with the wax.[39] Hence, wool wax cannot be built up by random combination of the known acids with the known alcohols. The indications are that diesters of hydroxy-acids form a considerable proportion of the total.

Summary of the Constituents of Wool Wax

The acidic and the alcoholic fraction each constitute about 50% of the wax.

Acidic fraction :	Approximate content, %
n-Acids :	
9 members, decanoic to hexacosanoic	7
iso-Acids :	
10 members, 8-methylnonanoic to 26-methylheptacosanoic	22
anteiso-Acids :	
11 members, 6-methyloctanoic to 28-methyltriacontanoic except 26-methyloctacosanoic	29
Hydroxy-acids :	
2 members, α-hydroxymyristic and α-hydroxypalmitic	7
Unidentified portion :	
unsaturated acids	15
unaccounted for	20
	—
Alcoholic fraction :	
Aliphatics :	
2 members, *n*-octadecanol, *n*-docosanol	3
Sterols :	
3 members, cholesterol	20
dihydrocholesterol	5
cholesta-3 : 5-dien-7-one	< 1

	Approximate content, %
*iso*Cholesterol :	
4 members, lanosterol	10
dihydrolanosterol	10
agnosterol	1
dihydroagnosterol	4
Hydrocarbons	< 1
Unidentified portion :	
straight-chain alcohols	15
diols	5
unaccounted for	25