CCLXXXVII. THE CONSTITUTION OF THE PRIMARY ALCOHOLS, FATTY ACIDS AND PARAFFINS PRESENT IN PLANT AND INSECT WAXES[1].

By ALBERT CHARLES CHIBNALL, STEPHEN HARVEY PIPER,
ALFRED POLLARD, ERNEST FRANK WILLIAMS
AND PREM NATH SAHAI.

From the Biochemical Department, Imperial College of Science and Technology, South Kensington, and the Wills Physical Laboratory, University of Bristol.

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INTRODUCTION.

By making use of the data for the primary alcohols, acetates, *n*-fatty acids and ethyl esters described in the previous paper [Piper *et al.*, 1934], and for the paraffins described in an earlier paper [Piper *et al.*, 1931] we are now in a position to unravel the composition of the mixtures of alcohols, acids and paraffins which occur in insect and plant waxes. Francis *et al.* [1930] examined with the aid of X-ray analysis a series of acids isolated from natural sources, and were able to show that the so-called arachidic, lignoceric, cerotic and montanic acids were mixtures of *n*-fatty acids, which accounted for the divergence of their melting-points from those of the pure synthetic acids to which they were supposed to correspond. In addition they were able to show by oxidation to the corresponding acids that the ceryl alcohol of Chinese wax and the melissyl alcohol of carnauba wax were not chemical entities but mixtures of primary alcohols. In the present paper we are able to amplify a little the conclusions of these authors with regard to the naturally occurring wax acids and completely to unravel the constitution of a large number of wax alcohols. Examination of some thirty samples of these alcohols has shown that they are all mixtures, but in certain cases one particular alcohol predominates to such an overwhelming extent that we have been able to isolate it in a state of undoubted purity. Thus *n*-triacontanol has been isolated from lucerne leaves [Chibnall *et al.*, 1933] and *n*-hexacosanol from blades of cocksfoot [Pollard *et al.*, 1931]. *n*-Octacosanol has been prepared from the blades of wheat [Pollard *et al.*, 1933]; in addition it was possible to show that at least three other alcohols were present in very small amount. The primary alcohol present in apple cuticle wax was shown, by fractionation of the derived paraffins, to be a mixture of the three above-mentioned alcohols [Chibnall *et al.*, 1931]. In this particular case the important point was definitely established that the neighbouring odd-number alcohols, *n*-heptacosanol and *n*-nonacosanol, were absent. Finally cocceryl alcohol, which is the sole alcoholic component of cochineal wax, has been shown to be 15-keto-*n*-tetratriacontanol [Chibnall *et al.*, 1934]. Since therefore all the

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[1] The melting-points recorded in this paper were obtained by the method described by Piper *et al.* [1931] and are corrected.

primary alcohols isolated from waxes contain an even number of carbon atoms, and the one mixture which we have been able completely to resolve contains no odd-number alcohols, we have accepted as a working hypothesis in unravelling the composition of the mixtures dealt with in the present paper that they are compounded solely of even-number alcohols.

With regard to the acid components of the waxes we find, in agreement with Francis *et al.* [1930], that they are all mixed. In no single instance have we obtained a product whose melting-point, titration value and melting-point of the ethyl ester suggested that one particular acid was present in such an overwhelming proportion that isolation of the pure acid by fractional crystallisation or ester distillation seemed at all probable. In one case only, that of cochineal wax, have we been able to resolve the acid mixture. Here the components were *n*-triacontanoic acid and 13-keto-*n*-dotriacontanoic acid, and separation was relatively easy as the solubility of the latter acid would be greatly increased by conversion into the oxime. Francis *et al.* [1930] were able to show that samples of acids supplied to them by Prof. Holde were pure, or nearly pure, *n*-tetracosanoic, *n*-hexacosanoic and *n*-octacosanoic acids respectively, but with the X-ray analytical data then at their disposal they could not definitely exclude the presence of odd-number acids from some of their mixtures from natural sources. A preliminary re-examination of their results however, which will be continued and is to be published in full elsewhere, leads to the belief that it is possible to describe all the mixtures they examined in terms of even-number chains.

There is evidence therefore satisfying the requirements of X-ray analysis, that the even-number *n*-fatty acids from C_{24} to C_{30} occur in waxes, and it appears that the acid mixtures usually obtained, like those of the closely related alcohols, are composed only of even-number *n*-fatty acids. By analogy with the lower *n*-fatty acids present in glycerides this result is perhaps to be expected, and discussion on the point might be considered superfluous, yet we would call attention to the fact that Gascard, one of the most careful workers in this field, has shown that the molecular weights of the well-known acids and alcohols isolated from beeswax (*cf.* Table IV below) suggest that all of them contain an odd number of carbon atoms, and it is only because we can now show that his preparations were not chemical entities, but mixtures, that we can state definitely that his conclusions must be erroneous, even though his chemical data remain undisputed. In the present paper we have accordingly interpreted all our acid and alcohol data on an even-number basis exclusively, and we feel that the results provide further proof, if need be, of the main assumption.

With regard to the paraffin components of the waxes a review of the literature to 1930 shows that the following have been isolated, the figures in brackets referring to the number of sources given for each individual member of the series. (Literature to 1930, *cf.* Wehmer [1931], Grün and Halden [1929].) *n*-Heptane (7), *n*-pentadecane (3), *n*-hexadecane (1), *n*-eicosane (1), *n*-heneicosane (1), *n*-docosane (3), *n*-tricosane (3), *n*-pentacosane (1), *n*-hexacosane (1), *n*-heptacosane (11), *n*-octacosane (1), *n*-nonacosane (2), *n*-triacontane (19), *n*-hentriacontane (26), *n*-dotriacontane (2), and *n*-pentatriacontane (15). Of these, the lowest two members, being readily volatile in steam, are found in essential oils, those with 16, 20, 21, 22, 23, are stated to occur in rose wax [Prophète, 1926], while the higher members, with 25–35 carbon atoms, are common constituents of plant and insect waxes.

The identification of these paraffins has been based on the only type of evidence available to workers at that time, *viz.* that supplied by combustion analysis for carbon and hydrogen, non-absorption of bromine or iodine, melting-point and occasionally by a determination of molecular weight. A full statement of the criteria necessary to determine the purity of a paraffin preparation has however since been given by Piper *et al.* [1931], which shows that the evidence quoted above is, by itself, quite insufficient to decide whether a given sample is a single paraffin or a mixture. Additional evidence based on crystal spacings and transition temperatures must be obtained before this can be stated with certainty.

In the account given below we discuss in some detail the composition of the various paraffin preparations which we have ourselves isolated from plant waxes and also of those isolated by other workers whose preparations have been kindly placed at our disposal for more complete identification. It will be seen that in all cases the products are mixtures, and that occasionally we are able to suggest their probable composition. In doing this we have acted upon the conclusion, drawn from our discussion given later, that all the paraffins which occur in waxes contain an odd number of carbon atoms.

In the following pages no attempt has been made to quote fully the literature appertaining to each individual wax, as this has been adequately collected by Grün and Halden [1929].

The data used in analysing the constituents of natural waxes are given in the previous paper, Piper *et al.* [1934]. The methods of applying the data are fully described in the same paper. It is a significant fact that within the range of wax constituents from C_{24} to C_{36} we are unable to suggest any synthetic mixtures of acids which melt at a temperature less than 2° above that of the corresponding mixture of alcohols. This statement is based on a careful examination of the melting-points of binary and ternary mixtures of acids up to C_{26} given by Francis *et al.* [1930], and those that we have recorded for binary and ternary mixtures of alcohols and of acids of chain lengths greater than C_{26}. Also it holds good for all possible combinations of both odd- and even-number chains. It draws further support from the wax analyses given below, in which the constituent acids and alcohols have been separated from paraffins *etc.* and rigidly purified by the methods outlined in previous papers, for there is no case in which the acids obtained by oxidation of the alcohol mixtures melt below this limit.

Among the numerous results of other workers quoted below however there are occasional examples in which the derived acids melt either at the same temperature or only 0·5–1·0° above that of the mixed alcohol. As the melting-points of acids are much more sensitive to the presence of impurities than those of the corresponding alcohols or esters, we prefer to consider that these low melting-points are due either to inert (*e.g.* paraffin) impurities present in the parent alcohol mixture which have passed through to that of the resulting acids, or to incomplete purification of the acid mixture from small amounts of unoxidised alcohols and/or soaps, rather than to the presence of any *iso*- or branched-chain acids which have never been detected by X-ray analysis. To keep down the amount of data given in the Tables which follow we have in most cases quoted only the upper limit of the melting-point and have omitted the setting-point.

Except when our results are at variance with those of previous workers we have not considered it necessary to give experimental details for the separation and purification of the components of each individual wax. As experience has been gained throughout the course of the work our methods have been modified in certain minor details, but in general these have followed closely those described by Chibnall *et al.* [1931], Pollard *et al.* [1931], Sahai and Chibnall [1932] and Chibnall *et al.* [1934]. In all cases the appropriate treatment for the separation of secondary alcohols and ketones has been applied, the primary alcohols and *n*-fatty acids have been separated *via* the calcium soaps, while paraffins have been freed from traces of other products by extraction at room temperature with cold petroleum, followed by a final treatment with sulphuric acid at 130°.

The first analysis, that of the mixed primary alcohols and *n*-fatty acids in lac wax, is described in some detail as the results are completely at variance with those of other workers. In subsequent cases our interpretation of the data is quoted without special comment.

The constituents of lac wax.

According to previous workers two different waxes are secreted by the lac insect (*Coccus lacca* Kerr; *Tachardia lacca* Kerr). The lac wax of commerce is obtained from stick-lac by heating it with water, whereby the wax separates from the mass and floats on the surface. On cooling the wax solidifies and is skimmed off. Only the lower-melting wax products are thus obtained however and it is more convenient to extract the washed stick-lac first with boiling alcohol, which dissolves the low-melting part of the wax, and to follow this with a second extraction with boiling benzene, which dissolves out a residual wax of very high melting-point.

The alcohol-soluble wax has been investigated by Tschirch and Schäfer [1926]. It melted at 80–81° and on saponification gave an alcohol "tachardiacerol," M.P. 78°, which analysis suggested was $C_{25}H_{52}O$, an acid "tachardiacerinic acid," M.P. 78°, which it was thought might be identical with "cerotic" acid, and a small amount of hydrocarbon, "tachardiacerin" melting at 54–55°.

The alcohol-insoluble, benzene-soluble wax has been investigated by Gascard [1921]. The product melted at 94°, and on saponification gave an alcohol "laccerol" and an acid "lacceroic acid." "Laccerol" after recrystallisation from benzene melted at 89° and on oxidation gave an acid which, after purification by repeated crystallisation, melted at 95–96°. It was clearly a primary alcohol, and analysis of the iodide suggested that it was *n*-dotriacontanol. Confirmation of this was sought by preparing the corresponding paraffin, which melted at 70–70·5°, the same temperature as that of the synthetic product prepared by him from cetyl iodide. "Lacceroic acid" on purification melted at 95°, was thought to be identical with the product obtained by oxidation of "laccerol," and gave a molecular weight on titration corresponding to *n*-dotriacontanoic acid.

We decided to investigate this wax as a possible source of *n*-dotriacontanol, for the melting-point of the alcohol quoted by Gascard was very close to that of our synthetic product and the derived acid melted 6–7° higher, an observation which we had till then taken as indicative of a high degree of purity. Yet the melting-point of his derived paraffin (70·5°) was higher than that of our synthetic *n*-dotriacontane (69·7°) and in particular that of his acetate (74·5°) was much higher than that of our synthetic product (72·7°), closer in fact to that of *n*-tetratriacontanol (75·8°). We suspected therefore that "laccerol," like the greater number of alcohols isolated from waxes, might be a mixture. Our analysis given below shows that this is indeed so; both the high- and low-melting waxes are mixtures of several homologues, and the lac insect undoubtedly synthesises all the even-number primary alcohols from C_{26} to C_{36}.

Preparation of the wax. For the preparation of the alcohol-soluble wax 250 g. of stick-lac were ground to a fine powder and exhaustively extracted at room temperature, first with water and then with 93 % alcohol. The residue was then digested with boiling 93 % alcohol and filtered hot. The alcohol-soluble wax (fraction 1) separated on cooling and was filtered off. For the preparation of the alcohol-insoluble, benzene-soluble wax 40 lbs. of stick-lac were exhaustively extracted with boiling alcohol, and the residue (390 g.) treated four times successively with boiling benzene (1400 ml.) for half an hour. The wax separated from the hot filtered solutions on cooling, and was filtered off (52 g.).

Fractionation of the alcohol-insoluble, benzene-soluble wax. The material was first digested for 6 hours with 95 % alcohol. The insoluble wax, which melted at 93·6–93·9°, was then digested 20 times successively with about 2 litres of benzene-alcohol (1 : 4), each digest being filtered hot. To keep the residual insoluble wax of uniform composition it was dissolved in boiling benzene after every third extraction and recrystallised by cooling the solution in the ice-chest. Each benzene-alcohol filtrate on cooling deposited a small amount of wax, which was filtered off. The melting-point of that from the first extract was 81·3°, from the eighth extract 91°, and from the twentieth extract 93·2°. These twenty small samples were collected (fraction 2; wt. 13 g.). The residue, after recrystallisation from benzene, melted at 94·2–94·7°. The treatment this material had so far received was similar to that of the "laccerol lacceroate" of Gascard [1921]; 3 g. were therefore set aside at this stage for comparison with his analytical data (fraction 3). The remainder was then treated a further 16 times successively with the benzene-alcohol. The melting-points of the samples of wax which separated on cooling the hot filtered extracts ranged from 93·4 to 94·4°, and the collected material weighed 9 g. (fraction 4). The residual material (fraction 4 A), after crystallisation from hot benzene, weighed 13·5 g. and melted at 95·1–95·3°.

Saponification of the various samples of wax. All the fractions were saponified by the method previously described [Chibnall *et al.*, 1934], the acids being separated as the calcium soaps. The crude primary alcohol from fraction 1 was then passed through the phthalate treatment to separate paraffin and other products [Chibnall *et al.*, 1931], and the purified sodium salts of the hydrogen phthalates were saponified [Pollard *et al.*, 1931]. The primary alcohol obtained was recrystallised from boiling acetone (charcoal). It melted at 82–82·2° and its acetate at 66·5°. The latter value is high, suggesting a quaternary mixture, so the alcohol was extensively fractionated by means of benzene-acetone at 37°. In this way a more insoluble product melting at 83·4–83·6° (fraction 6), and a more soluble product melting at 81·8–82° (fraction 5) were obtained. The calcium soaps from fraction 1 gave only water-soluble products, and no fatty acids were obtained at all. As this finding is contrary to that of Tschirch and Schäfer [1926] we saponified a sample (50 g.) of commercial lac wax and investigated the calcium soap fraction. Again we found that all the acidic products were water-soluble. There can be no doubt therefore that our samples of both stick-lac and commercial lac wax contained no "tachardiacerinic" acid, so that fraction 1 contained no wax esters at all and the alcohol present was free.

Fractions 2–4 were saponified, but the phthalate treatment was omitted. In each case the resulting alcohols and acids were crystallised first from boiling acetone (charcoal) and then twice from acetone at 37°. Fraction 2 yielded an alcohol, M.P. 86·9° (fraction 7), and an acid, M.P. 91·4° (fraction 8); fraction 3 an alcohol, M.P. 89° (fraction 9), and an acid, M.P. 93·6° (fraction 10); fraction 4 an alcohol, M.P. 88·3° (fraction 11), and an acid M.P. 92·4° (fraction 12).

Fraction 5 was treated in a similar way, but the alcohol was further fractionated. The material was extracted repeatedly at 40° with acetone until the residue weighed about 1·5 g. The acetone extracts were cooled in the ice-chest, the separated material was filtered off, collected, and 1 g. recrystallised five times successively from a mixture of 140 ml. of acetone and 30 ml. of benzene at 37°. The product thus obtained melted at 89·6–89·8° (fraction 13). The residual 1·5 g. mentioned above, M.P. 89–89·2°, was recrystallised three times successively from 100 ml. of acetone-benzene (4 : 1), giving 0·5 g. of material melting at 90·4–90·6° (fraction 14). The acids obtained from the calcium soaps were crystallised from boiling acetone (charcoal) and then recrystallised twice from acetone at 37° (fraction 15). The melting-point was 95·5–95·7°.

Analysis of the various fractions of primary alcohol and n-*fatty acids.* The acetates, derived paraffins and derived acids of the primary alcohols were made by our standard procedure [Pollard *et al.*, 1931]. As all these alcohols are shown later to be mixtures, it follows that drastic purification of the derived acid will bring about a change in composition so that the melting-points of the two products are not comparable (*cf.* criticism of Gascard given below). Our procedure throughout has been to crystallise the crude acid once from boiling acetone and then again from acetone at 37°. We have called attention in an earlier paper to the fact that wax acids and alcohols which are separated and purified by the methods we adopt often exhibit when melted a mist which obscures the sharpness of the melting-point determination. This is due to the presence of a small amount of soap, which can be removed by extraction with acetone at 40°. A very small insoluble residue is always obtained. Derivatives such as acetates, esters and paraffins can be cleaned in a similar way by extraction at room temperature with low-boiling petroleum.

In the following account we examine each of the fractions mentioned above in some detail, the identifications having been carried out as described in the previous paper [Piper *et al.*, 1934]. Details of the alcohol fractions are given in Table I and of the acid fractions in Table II.

Table I. *Constitution of the primary alcohols of lac wax.*

	Alcohol				Derived acid			
Fraction No. and composition of suggested mixture	M.P. °C.	Crystal spacings Series No.	Crystal spacings Å.	Acetate M.P. °C.	M.P. °C.	Crystal spacings Series No.	Crystal spacings Å.	Derived paraffin M.P. °C.
5	82·0	B 384	79·5	65·6	85·3	B 386	71·6	62·3
20 % 26 + 40 % 28 + 40 % 30	82·3	B 338	78·9	65·6	84·8	B 332	71·7	62·1
6	83·6	B 385	82·9	68·5	86·4	B 387	79·2	65·4
Equimolar 28 + 30 + 32	84·0	B 390	81·0	68·5	87·0	B 333	77·6	65·8
7	86·9	B 362	86·5	72·5	89·7	B 364	83·1	68·8
40 % 30 + 40 % 32 + 20 % 34	86·9	—	86·3	72·1	89·7	—	80·5	68·9
11	88·3	B 361	88·8	73·3	92·1	B 363	81·6	70·3
20 % 30 + 40 % 32 + 40 % 34	88·2	—	89·4	73·2	91·1	—	80·5	70·0
13	89·8	B 345	74·5 90·4	74·8	94·2	B 359	81·1	71·9
35 % 32 + 65 % 34	90·0	—	90·4	74·8	94·5	—	80·5	71·7
14	90·6	B 346	75·1 91·4	75·4	95·6	B 360	? 74·3	72·3
20 % 32 + 80 % 34	90·7	—	91·0	75·3	95·8	—	80·8 73·8	72·2

Alcohol fraction 5. This corresponds to a high-melting "ceryl" alcohol. In this case we prepared a synthetic mixture of the suggested composition, and it will be seen that the correspondence throughout is good.

Alcohol fraction 6. This fraction is typical of "montanyl" alcohol. Again we have prepared a synthetic mixture of the suggested composition, and the only noticeable differences between the two are the high spacing values of the fraction. This means the presence of a small amount of C_{34}, and a corresponding lowering of the suggested amounts of the other components.

Fractions 7, 11, 13, 14. Fractions 7 and 11 are typical "myricyl" and "melissyl" alcohols respectively. The extrapolated data give compositions which are satisfactory except for the high values of the acid spacings. These can be accounted for by the addition of a small amount of C_{36} to each mixture. An interesting point is the unusually strong C spacing found in fraction 14. The B spacing is also present on the plate but is too diffuse to be measurable. The appearance of both B and C spacings in the alcohols of fractions 13 and 14 corresponds to the behaviour of binary mixtures, and suggests that the contamination by C_{36} must be small.

Table II. *Constitution of the fatty acids of lac wax.*

Fraction No. and composition of suggested mixture	M.P. ° C.	Crystal spacings		Mol. wt. by titration
		Series No.	Å.	
8	91·4	B 366	80·3	498
12	92·4	B 365	80·1	492
20 % 30 + 40 % 32 + 40 % 34	91·1	—	80·5	486
10 % 30 + 45 % 32 + 45 % 34	—	—	—	490
15	95·7	B 350	80·5 73·9	495
20 % 32 + 80 % 34	96·0	—	80·5 74·0	503

Acid fractions 8 and 12. Both have a spacing requiring 20 % or more of C_{34} if they are ternary mixtures. If binary mixtures the proportion of C_{34} would have to be 60 % or more, and this would require much higher melting-points than are found. Agreement is on the whole satisfactory and shows the presence of 10–20 % of C_{30}, but the suggested molecular weight is slightly lower. The observed values were obtained by titration in benzene-alcohol (1 : 3) at the boiling-point to the first pink colour of phenolphthalein, and duplicate values differed by 5 units, so that too great stress need not be paid to small discrepancies between the observed and suggested values.

Acid fraction 15. The suggested composition appears good. For binary mixtures of C_{28} and C_{30} the B and C spacings occurred at 20 % C_{28} + 80 % C_{30} agreeing with their simultaneous appearance in this fraction. The molecular weight observed in this case is rather low for the mixture suggested, but the melting-points are in good accord.

Our finding that the high-melting wax contains mixtures of alcohols and acids with 30, 32, 34 and 36 carbon atoms respectively, shows that Gascard's "laccerol" and "lacceroic" acid were not chemical entities. His quoted melting-points are: alcohol 89°, acetate 74·5°, paraffin 70·5° and derived acid 95–96°. Our fraction 9, which we consider is similar to his, gave corresponding melting-points of 89, 73·8, 70·7 and 92·3° respectively. Gascard definitely states that his derived acid was purified by repeated crystallisation, and there appears to be no doubt that he has in this way shifted the composition of his acid mixture towards the longer-chain, higher-melting products corresponding to the derived acid from our own fraction 14.

No paraffin was obtained from fraction 1 after the phthalate treatment. Tschirch and Schäfer [1926] prepared "tachardiacerin" by drastic destruction of the crude alcohol-soluble wax with sulphuric acid. It melted at 54–55° and gave on analysis C 88·8, H 11·3 %. These values are erroneously quoted as agreeing with those of $C_{25}H_{52}$, which are C 85·1, H 14·9 %.

Summarising our results we find that alcohol-soluble or commercial lac wax contains no fatty acid or paraffin, and is a simple mixture of the even-number primary alcohols from C_{26} to C_{34}. The alcohol-insoluble, benzene-soluble, wax is a mixture of esters containing the even-number primary alcohols from C_{30} to C_{36} and the even-number *n*-fatty acids from C_{30} to C_{34}.

Constituents of various insect waxes.

Cochineal wax (*coccerin*), from *Coccus cacti*, has a relatively simple constitution, and consists of 15-keto-*n*-tetratriacontanol (cocceryl alcohol), *n*-triacontanoic acid and 13-keto-*n*-dotriacontanoic acid [Chibnall *et al.*, 1934].

Chinese insect wax, from *Coccus ceriferus*, has been repeatedly analysed and shown to consist of "ceryl cerotate." The constitution of the "ceryl" alcohol was discussed in a previous paper [Pollard *et al.*, 1931], and it was shown to be neither C_{26} nor C_{27} as earlier workers had assumed, but a mixture of primary alcohols which the data of Francis *et al.* [1930] suggested was C_{26} and C_{28} with a higher homologue. We have analysed a sample of the "ceryl" alcohol from this wax, and the results are given in Table III. The major constituents are clearly C_{26} and C_{28}, but the derived paraffin gives a diffuse X-ray picture and poor transition temperatures, showing the presence of three or more homologues, while the crystal spacing of the derived acid definitely shows the presence of C_{30}. The composition appears to be about 40 % C_{26} + 40 % C_{28} + 20 % C_{30}. The data of Francis *et al.* [1930] show clearly that the "cerotic" acid of this wax is a mixture of C_{26}, C_{28} and C_{30}.

Table III. *Chinese insect wax. Constitution of the "ceryl" alcohol.*

	Alcohol				Derived acid			Derived paraffin		
		Crystal spacings		Acetate		Crystal spacings			Crystal spacings	
	M.P. ° C.	Series No.	Å.	M.P. ° C.	M.P. ° C.	Series No.	Å.	M.P. ° C.	Series No.	Å.
"Ceryl" alcohol	79·7	C 45	74·7	62·0	83·2	B 351	69·85	59·5	C 44	36·7
Corresponding mixtures:										
50 % 26 + 50 % 28	80·2	B 252	73·5	62·5	83·5	—	65·5	59·1	B 97	36·7
40 % 26 + 60 % 28	80·9	B 253	74·6	63·0	84·2	—	66·5	59·7	B 46	36·8
40 % 26 + 40 % 28 + 20 % 30	80·4	B 339	75·7	63·8	83·2	B 334	71·6	60·3	—	—

Psylla wax, from *Psylla alni*. This very rare wax, M.P. 96°, has been investigated by Sundwick [1901]. On hydrolysis with hydrobromic acid it gave "psyllostearic" acid, M.P. 94–95°, and "psyllostearyl" alcohol, M.P. 68–70°. The latter was considered to be impure, and to obtain a purer derivative the benzoate, M.P. 68–69°, was prepared. Carbon and hydrogen analysis of the wax suggested that the products contained 33 carbon atoms, but there is little doubt that the psyllostearyl alcohol was a mixture of alcohols and paraffin (*cf.*

incarnatyl alcohol). Heiduschka and Garies [1919] state that the benzoate of their "mellisyl" alcohol from carnauba wax, which we show later is a mixture of C_{30}, C_{32} and C_{34}, melts at 69·5°. Psyllostearic acid is undoubtedly a mixture corresponding to the lac acids given in Table II.

Beeswax, from *Apis mellifica*. Numerous attempts have been made to determine the constitution of the various constituents of this wax; the most extensive are those of Gascard and Damoy [1923] and Damoy [1924], whose data for the primary alcohols are set forth in Table IV, together with those of what we consider to be the corresponding mixtures.

Table IV. *Probable constitution of the primary alcohols isolated from beeswax. (Data of Gascard and Damoy [1923].)*

	Alcohol M.P. ° C.	Acetate M.P. ° C.	Derived acid M.P. ° C.	Derived paraffin M.P. ° C.
"Neoceryl" alcohol (C_{25})	75·5	59·3	77·8	—
Suggested mixture: 40 % 24 + 40 % 26 + 20 % 28	76·1	59·0	79·0	—
"Ceryl" alcohol (C_{27})	80·0	—	82·0	59·5
Suggested mixture: 50 % 26 + 50 % 28	80·2	—	83·5	59·1
"Montanyl" alcohol (C_{29})	84·0	68·2	86·8	—
Suggested mixture: 40 % 28 + 40 % 30 + 20 % 32	83·8	68·0	86·6	—
"Myricyl" alcohol (C_{31})	87·0	72·5	90·0	69·0
Suggested mixture: 40 % 30 + 40 % 32 + 20 % 34	86·9	72·1	89·7	68·7

There is no doubt that their "neoceryl," "ceryl," "montanyl" and "myricyl" alcohols (which were considered to have 25, 27, 29 and 31 carbon atoms respectively) are all mixtures, and that the wax contains all the even-number primary alcohols from C_{24} to C_{34} inclusive. The free acids isolated by them, "neocerotic," "cerotic," "montanic" and "melissic" acids melted at the same temperatures as those derived from the four alcohols and were considered to have corresponding structures. All the even-number *n*-fatty acids from C_{24} to C_{34} are therefore probably present. The paraffins were fractionated by means of benzene-alcohol, and C_{25}, M.P. 54–54·5°; C_{27}, M.P. 59·5°; C_{29}, M.P. 64·5° and C_{31}, M.P. 68·4° were obtained, the second and fourth in largest amount. Our own results with mixed paraffins, which have been controlled by X-ray analysis and observations on transition temperatures, suggest that pure products cannot be obtained from such a mixture by fractional crystallisation, but we see no reason to doubt that the four paraffins mentioned by them were present in the wax.

South Brazilian wild bee wax, from *Melipona* sp., has been examined by Godamer [1917] who found an alcohol, M.P. 92–94°, "mellisyl" alcohol, M.P. 85–86°, "ceryl" alcohol, M.P. 79°, and a crude paraffin, M.P. 55°. Other less well-defined products, one of which appeared to be a C_{27} alcohol of the paraffin series with two double bonds, were also isolated. The saturated alcohols suggest a mixture similar to that of lac wax, containing all the even numbers from C_{26} to C_{34}.

Ghedda wax (*East Indian insect wax*), from *Apis dorsata*, has been examined by Lipp and Casimir [1919]. "Ceryl" alcohol, M.P. 76°, "cerotic" acid, M.P. 78° and "Ghedda" acid, M.P. 94·5–95°, considered to be C_{34}, were obtained. The results suggest a mixture of even-number alcohols from C_{24} to C_{30}, and of even-number acids from C_{24} to C_{34}. Paraffins melting at 59–59·5° and 68–68·5° were obtained after a long fractional crystallisation, probably consisting in large part, as they suggest, of C_{27} and C_{31} respectively.

Bumble-bee wax, M.P. 75°, from *Bombus lapidarius*, was examined by Sundwick [1907], who isolated "hummel" alcohol, M.P. 69–69·5°. This alcohol was fairly readily soluble in the usual solvents, yet the carbon and hydrogen analysis suggested that it was C_{33}. The product appears to be a mixture of "ceryl" alcohol and paraffin (*cf.* incarnatyl alcohol).

Leaf cuticle waxes.

These waxes are cuticle excretions and can be prepared by immersing the leaves in hot water, when the wax separates and floats to the surface. All of them are of possible commercial importance for this reason.

Carnauba wax. The primary alcohol was investigated by Heiduschka and Garies [1919], and from the analytical data of several derivatives they concluded that it was "melissyl" alcohol with 31 carbon atoms, thus differing from the "myricyl" alcohol of beeswax, which they considered had 30 carbon atoms. Melting-points quoted by them are: alcohol 87·5°, acetate 74°, derived acid 90°, in fair agreement with the mixture 20 % C_{30} + 40 % C_{32} + 40 % C_{34}, which has values of 88·2, 73·2 and 91·1° respectively. The presence of a small amount of lower alcohols is, however, suggested and this receives confirmation from the work of Gottfried and Ulzer [1926], who fractionated the acetate by distillation *in vacuo* and obtained as the main product (1) an acetate melting at 74° which gave an alcohol melting at 88°, and (2) a small amount of acetate melting at 64·5° which gave an alcohol melting at 79°. The latter is typical of "ceryl" alcohol consisting of 40 % C_{26} + 40 % C_{28} + 20 % C_{30} (63·8, 80·4°). The primary alcohol of the wax therefore contains all the even numbers between C_{26} and C_{34}, the higher ones predominating. Similar conclusions as to the constitution of the latter alcohols can be drawn from the data of Francis *et al.* [1930].

The wax fatty acid has received less attention, but there seems to be general agreement that it is similar to that obtained on oxidation of the alcohol. The melting-point is given as 90° and that of the ethyl ester as 73°, so that the main components are undoubtedly C_{30}, C_{32} and C_{34}. By long fractionation Stürcke [1884] isolated a low-melting fraction (79°) which he considered to be "cerotic" acid, so that small amounts of C_{26} and C_{28} are probably present as well.

Both Stürcke and Gottfried and Ulzer isolated a small amount of paraffin, M.P. 58·5–59°, which they considered to be *n*-heptacosane.

Candelilla wax. This wax has been repeatedly analysed. The preliminary separation of the constituents of a commercial sample yielded about 20 % of

Table V. *Constitution of the primary alcohol and* n-*fatty acid of Candelilla wax.*

	Alcohol	Alcohol: Crystal spacings			Acid	Acid: Crystal spacings		
	M.P. °C.	Series No.	Å.	Acetate M.P. °C.	M.P. °C.	Series No.	Å.	Derived paraffin M.P. °C.
Alcohol	86·2	B 340	88·6	70·0	89·0	B 354	83·0	67·8
Suggested mixtures:								
Equimolar $C_{30}+C_{32}+C_{34}$	87·1	—	87·5	72·5	90·0	B 329	81·6	69·7
40 % C_{30} + 40 % C_{32} + 20 % C_{34}	86·9	—	86·7	70·9	89·7	—	81·0	68·7
Equimolar $C_{30}+C_{32}$	86·7	—	83·5	70·8	90·2	—	75·0	67·8
Fatty acid	—	—	—	—	89·2	B 353	81·4	—
Suggested mixture:								
Equimolar $C_{30}+C_{32}+C_{34}$	—	—	—	—	90·0	B 329	81·6	—

resin, 50–60 % of paraffin, 15 % of "mellisic" acid, part free and part as ester, and 5 % of "myricyl" alcohol [*cf.* Buchner, 1918]. Secondary alcohols and ketones were absent.

The results of our analysis of the primary alcohol and fatty acid are given in Table V. The paraffin derived from the alcohol gave a fairly definite transition on heating at 60·0–60·5°, showing that the major constituents are about equimolar $C_{30}+C_{32}$. The spacings of both the alcohol and its derived acid however show the presence of at least 10–15 % of C_{34}, so it is probable that a small amount of C_{28} is also present, for the melting-point of the derived paraffin suggests a mean molecular weight corresponding to C_{31}. The suggested composition is therefore 35–40 % of both C_{30} and C_{32}, with 10–15 % of both C_{28} and C_{34}. The wax fatty acid clearly has a corresponding composition. The "oxylactone" of Meyer and Soyka [1913] which melted at 88–88·5° was undoubtedly a wax ester of the above products.

Buchner [1918] found that the paraffin of the wax melted at 68° and considered that it was *n*-hentriacontane. Meyer and Soyka [1913], however, found a higher melting-point, 71°, and considered that their product was *n*-dotriacontane, as there was no depression in melting-point when it was mixed with the synthetic product prepared from cetyl iodide. We have already called attention to the unreliability of such evidence in deciding whether a preparation of paraffin is a chemical entity or a mixture. Our own sample of paraffin, after treatment with sulphuric acid to free it from traces of impurity, melted at 67·8–67·9. The transition temperature on heating was indistinct at 61·5–62°, and the product was clearly a mixture. The material was fractionated from light petroleum and from benzene-alcohol, when a small higher-melting fraction (B) was obtained melting at 68·0° and a main fraction (A) which was very nearly pure C_{31}. Details of the analyses are given in Table VIII.

Raphia wax, from *Raphia ruffia,* was considered by Haller [1907] to consist chiefly of raphia alcohol $C_{20}H_{42}O$, M.P. 80°. A sample of this wax was kindly supplied by the Imperial Institute, and was examined by Dr R. C. Jordan. On saponification it gave a large amount of alcohol, a very small amount of fatty acid and no secondary products or paraffins. Data for the alcohol given in Table VII later suggest that it is a mixture of 40 % 28 + 40 % 30 + 20 % 32.

Pisang (*Gedang*) *wax,* from *Musa sapientum.* This wax was examined by Greshoff and Sack [1901] who found pisang alcohol, M.P. 78°, and pisangceryl acid, M.P. 71°, as ester. A sample of this wax was kindly supplied by the Director of the Botanical Gardens, Buitenzorg, Java. On saponification it gave a large amount of primary alcohol, a small amount of fatty acid that was not examined and no secondary products or paraffins. Data for the alcohol given in Table VII later show that it is a mixture of about 20 % C_{28} + 40 % C_{30} + 40 % C_{32}.

Fruit and seed coat cuticle waxes.

Apple cuticle wax. This has been examined in great detail by Chibnall *et al.* [1931], and shown to consist of *n*-heptacosane, *n*-nonacosane, *d*-*n*-nonacosan-10-ol, primary alcohol and fatty acid. The composition of the primary alcohol was determined by fractionation of the derived paraffin and definite proof was obtained that C_{26}, C_{28} and C_{30} were present, and that C_{27} and C_{29} were absent. Other data obtained from this alcohol, *i.e.* alcohol, M.P. 81·0°; acetate, M.P. 64·8°; derived acid, M.P. 83·5°; derived paraffin, M.P. 61·5°; are in fair agreement with those of equimolar $C_{26}+C_{28}+C_{30}$, *i.e.* 80·7°, 64·4°, 83·4° and 61·8° respectively. The fatty acid, M.P. 82·2–82·8°, was fractionated by means of acetone at 37°

and gave two samples: (1) M.P. 81–81·5° with a crystal spacing (Series No. B 356) of 70·8 Å. and (2) M.P. 85·6–85·8°, with a crystal spacing (Series B 355) of 75·4 Å. These melting-points and spacings suggest mixtures containing C_{26}, C_{28}, C_{30} and C_{32}.

Wax of American cotton. This wax has been exhaustively studied by Fargher and Probert [1924]. The alcohols were fractionated through their acetates, whereby "montanyl" alcohol, $C_{28}H_{58}O$, "gossypyl" alcohol, $C_{30}H_{62}O$, and two others considered to be $C_{32}H_{66}O$ and $C_{34}H_{70}O$ were obtained. Derived acids were prepared from them, and the complete melting-point data are reproduced in Table VI, together with those of mixtures which we suggest very closely represent their composition. The even-number primary alcohols from 28 to 34 are indeed present, but none of the products isolated shows any approach to purity, for all of them are at least ternary mixtures. A series of free acids, with melting-points and titration values corresponding to those derived from the alcohols, was also present, as also were lower-melting products, corresponding to "ceryl" alcohol, "cerotic" acid and "carnaubic" acid $C_{24}H_{48}O_2$. The latter acid melted at 72–73° and its ethyl ester at 52–53°, suggesting a composition approximating to equimolar $C_{22}+C_{24}+C_{26}$. Cotton wax therefore, like beeswax and lac wax, contains all the even-number primary alcohols and *n*-fatty acids from C_{24} to C_{34}, with possibly shorter-chain products.

Table VI. *Probable constitution of the primary alcohols of American cotton wax.* (*Data of Fargher and Probert [1924]*.)

	Alcohol M.P. ° C.	Acetate M.P. ° C.	Derived acid M.P. ° C.
Montanyl alcohol (C_{28})	83·5	67–67·5	83·5–84
Suggested mixture: 40 % C_{28} + 40 % C_{30} + 20 % C_{32}	83·8	68	86·6
Gossypyl alcohol (C_{30})	85·0	69–69·5	86·5
Suggested mixture: 20 % C_{28} + 40 % C_{30} + 40 % C_{32}	85·4	69·5	87·9
Alcohol (C_{32})	87–87·5	72·5	88–89
Suggested mixture: equimolar C_{30} + C_{32} + C_{34}	87·1	72·7	90
Alcohol (C_{34})	88·5–89	74·5	90–91
Suggested mixture: 50 % C_{32} + 50 % C_{34}	89·4	74·5	93·1
" 20 % C_{30} + 40 % C_{32} + 40 % C_{34}	88·2	73·2	91·1

Fargher and Probert [1923] isolated two paraffins which were considered to be C_{30} and C_{31}, while Power and Chesnut [1925] isolated a product, M.P. 62°, which was considered to be C_{30}. Dr Chesnut placed a small amount of the latter product at our disposal for more complete identification. It melted at 61·7–61·9°, and set at 61·2°, the material separating as an amorphous mass with no trace of crystalline form. No transition temperatures on either heating or cooling were observed, and the material was clearly a mixture of four or more paraffins.

Various leaf waxes.

The following waxes are not cuticle excretions similar to carnauba wax mentioned above, but are integral parts of the general fat phase of the leaf cells.

Brussels sprout (Brassica oleracea v. gemmifera). This wax has been investigated by Sahai and Chibnall [1932] and contains *n*-nonacosane, *n*-hentriacontane, *n*-nonacosan-15-one, *n*-nonacosan-15-ol, primary alcohol and fatty acid. The primary alcohol has been further examined (Table VII). The transition temperatures of the derived paraffin are low and too diffuse for the mixture 40 %

Table VII. *Composition of primary alcohols present in various leaf waxes.*

Source of alcohol or composition of suggested mixture	Alcohol M.P. ° C.	Alcohol Crystal spacings Series No.	Alcohol Crystal spacings Å.	Acetate M.P. ° C.	Derived acid M.P. ° C.	Derived acid Crystal spacings Series No.	Derived acid Crystal spacings Å.	Derived paraffin M.P. ° C.
Brussels sprout	80·8	B 341	74·6	—	84·2	B 252	73·1	59·7
40 % 26 + 60 % 28	80·9	B 278	74·6	—	84·4	—	67·0	59·5
Cactus	82·4	—	—	—	85·7	—	—	63·7
Equimolar $C_{28} + C_{30}$	83·6	—	—	—	87·3	—	—	63·6
White mustard	85·0	—	—	—	87·8	—	—	66·4
20 % 28 + 40 % 30 + 40 % 32	85·6	—	—	—	87·9	—	—	66·5
Wild white clover	83·7	—	—	—	87·3	—	—	63·7
50 % 28 + 50 % 30	83·6	—	—	—	87·3	—	—	63·6
Cluytia similis								
Clutyl alcohol	81·5	—	—	64·0	85·8	—	—	—
30 % 26 + 70 % 28	81·5	—	—	63·5	86·0	—	—	—
Raphia ruffia								
Raphia alcohol	83·4	—	—	67·9	86·2	—	—	—
40 % 28 + 40 % 30 + 20 % 32	83·8	—	—	68·0	86·6	—	—	—
Musa sapientum								
Pisang alcohol	85·2	—	—	69·5	87·6	—	—	—
20 % 28 + 40 % 30 + 40 % 32	85·6	—	—	69·5	87·9	—	—	—

C_{26} + 60 % C_{28} suggested by the melting-point and crystal spacings of the alcohol, showing the presence of other homologues. The crystal spacing of the derived acid definitely shows the presence of C_{32}, and the composition of the alcohol is probably 40 % C_{26}, 40 % C_{28}, 20 % of C_{30} and C_{32}. The fatty acid melted at 79–79·5°, but only a very small amount was isolated, and none was available for purification by our present methods. It was undoubtedly the usual "cerotic" acid mixture, so that the presence of C_{26} and C_{28} and probably of C_{24} and C_{30} can be inferred.

Cabbage (Brassica oleracea *v.* capitata). This wax was examined by Channon and Chibnall [1929] and shown to contain *n*-nonacosan-15-one and a paraffin, M.P. 62·7–62·8°, identified at the time as *n*-nonacosane. It is now known that this melting-point is too low for the pure substance. After treatment with sulphuric acid in the usual way the product had the melting-point, transition temperatures and crystal spacing given in Table VIII, showing that it was undoubtedly a mixture of about 95 % C_{29} and 5 % C_{31}. As the original wax had an acetyl value of 12, it is probable that small amounts of primary alcohol and *n*-nonacosan-15-ol were also present.

Swede turnip (Brassica campestris *L.*). This wax yielded a very small amount of primary alcohol, a paraffin, a secondary alcohol but no ketone. The paraffin (Table VIII) was C_{29} with about 5 % C_{31}. The secondary alcohol melted at 83·1° and was identified as *n*-nonacosan-15-ol.

White mustard (Brassica alba *Boiss*). This wax was examined by Dr J. A. B. Smith. It contained neither secondary alcohol nor ketone. Data for the primary alcohol given in Table VII show that it is a mixture of about 20 % C_{28} + 40 % C_{30} + 40 % C_{32}. The fatty acid on purification melted at 84·2° and on titration gave a molecular weight of 440, showing that it was a mixture of approximately 20 % C_{26} + 40 % C_{28} + 40 % C_{30}. The paraffin melted at 66–66·2° and would have been considered by earlier workers to be C_{30}. It showed a definite transition at 58–58·5° on heating, and was undoubtedly a mixture of 35 % C_{29} and 65 % C_{31}.

Table VIII. *Composition of paraffin isolated from various plants.*

Source of paraffin and composition of suggested mixture	Heating transition temperature ° C.	M.P. ° C.	S.P. ° C.	Cooling transition temperature ° C.	Crystal spacings Series No.	Crystal spacings Å.
Cabbage	57·1–57·3	63·6–63·8	63·4	56·1	C 88	39·13
95 % 29 + 5 % 31	57 –57·5	63·6–63·8	63·2	55·4	C 97	39·15
Swede turnip	57 –57·2	63·6–63·8	63·4	55·4	B 379	39·34
95 % 29 + 5 % 31	57 –57·5	63·6–63·8	63·2	55·4	C 97	39·15
Cactus fraction A	72 –72·5	76·0–76·1	75·8	72·0	B 371	49·2
50 % 35 + 50 % 37	72 –72·5	75·9–76·0	—	—	—	49·2
Cactus fraction B	71·0–71·5	74·5–74·7	74·1	70·5	B 370	47·1
95 % 35 + 5 % 37	71·0–71·5	74·5–74·7	—	—	—	47·1
Cactus fraction C	65·0–65·5	72·1–72·3	71·9	64·5	B 381	47·0
70 % 33 + 30 % 35	66·0	72·3	—	—	—	46·7
Tobacco fraction A	—	68·4–68·6	68·2	—	B 376	42·8
80 % 31 + 20 % 33	62·0	68·8	—	60·0	—	42·8
Tobacco fraction B	61·0–61·5	67·6–67·8	67·4	60·0	B 377	42·2
90 % 31 + 5 % 29 + 5 % 33	61·0	67·8	—	60·0	—	About 42
Cannabis indica						
Fraction A	57·0–57·8	65·9–66·0	65·3	56·5	B 1	41·3
40 % 29 + 60 % 31	57·5–58·0	65·8–66·0	65·5	56·55	C 100	41·2
Cannabis indica						
Fraction B	56·5–57·0	64·7–64·9	64·5	55·5	B 3	40·3
70 % 29 + 30 % 31	56·5–57·0	64·6–64·7	64·1	55–54	C 103	40·55
Candelilla wax						
Fraction A	62·0–62·2	67·7–67·8	67·4	61·4	B 342	41·46
C_{31}	62·2–62·5	67·7–67·8	67·3	61·8	C 32	41·55
Fraction B	62·0–62·2	67·9–68·0	67·6	61·1	B 372	42·0
95 % 31 + 5 % 33	62·0–62·2	67·9–68·0	67·4	61·3	—	42·0

Cactus (Opuntia *sp.*). This plant is the host of the cochineal insect, and it seemed to us of interest to find out if the wax contained any of the unique constituents found in the wax of the insect. Dr Forrest Shreve, of the Desert Laboratory, Tucson, Arizona, kindly provided us with 7·4 kg. of dried plant material. The light petroleum extract (66 g.) gave 18·3 g. of crude wax, and this on saponification 4·5 g. of crude fatty acids and 11·7 g. of unsaponifiable material which consisted of primary alcohol and paraffin. Data for the primary alcohol are given in Table VII. The transition temperatures of the derived paraffin were fairly sharp and about 1·5–2° below those of the suggested binary mixture, indicating the presence of only small amounts of homologues. The probable composition appears to be C_{28} and C_{30} predominating, with small amounts of C_{26} and C_{32}. The naturally occurring paraffin melted at 74·2–74·5° and would have been considered by earlier workers to be C_{35}. Fractionation by means of cold petroleum however gave three products whose melting-points and crystal spacings are given in Table VIII. Fraction A melts slightly above C_{36}, but the transition temperatures are 2° too low and the crystal spacing 1·7 Å. too high for this paraffin. But the transition temperatures, crystal spacings, and general character of the X-ray photograph are in excellent agreement with equimolar $C_{35}+C_{37}$. Fraction B is clearly C_{35} with about 5 % of C_{37}, while fraction C which melts slightly below C_{34} has transition temperatures and crystal spacings which will not fit those of a mixture $C_{34}+C_{35}$, but are in good agreement with 70 % C_{33} + 30 % C_{35}. These three X-ray photographs are excellent examples of those required for the suggested mixtures, showing 7–9 sharp orders, and it may be accepted with confidence that the cactus paraffin definitely contains no even-number component. The wax fatty acids were separated into a large fraction melting at 67°, having a molecular weight corresponding to C_{22}, and a small fraction melting at 87°.

Tobacco (Nicotiana tabacum). Thorpe and Holmes [1901] found that ether extraction of cured tobacco leaves gave a clean white wax, M.P. 63·3–63·8°, which was unchanged on saponification. Carbon and hydrogen analysis showed that it was a paraffin, and by fractional crystallisation from ether it was separated into two products, melting at 67·8–68·5° and 59·3–59·8° respectively, which were identified as C_{31} and C_{27}. Dr R. C. Jordan has repeated this work. Separation into high- and low-melting paraffin fractions was relatively easy, and three samples were selected for further analysis. The data given in Table VIII show that pure products have not been obtained. Fractions A and B show that C_{33}, C_{31}, and perhaps C_{29}, are present. Fraction C, melting at 59·5°, showed only indistinct transitions and gave an X-ray photograph too poor for interpretation. It was a mixture of at least 3 or 4 paraffins, presumably containing C_{29}, C_{27} and C_{25}.

As the wax from cured tobacco contained no primary alcohol, fatty acid or secondary products, we suspected that these might have been metabolised during the process of curing. If this be so then analysis of the leaf wax at various stages might throw light on the enzymic breakdown of long-chain aliphatic substances, and we felt it worth while to analyse the wax of green tobacco leaves. Dr H. B. Vickery, of the Connecticut Agricultural Experimental Station, kindly placed at our disposal the light petroleum extract from 4·5 kg. of air-dried green Connecticut tobacco. The extract (400 g.) gave 20·8 g. of crude wax, and Dr J. A. B. Smith showed that this consisted almost entirely of mixed paraffins melting at 63·3–63·8° as before.

Wild white clover (Trifolium pratense *L.*). This wax gave only primary alcohol and fatty acid. Data for the primary alcohol are given in Table VII. The derived paraffin had a fairly sharp transition temperature on heating at 54–54·4°, confirming the suggested composition. The fatty acids melted at 83° and had a molecular weight by titration of 420, suggesting approximately equimolar $C_{26}+C_{28}+C_{30}$.

Spinach (Spinacea oleracea). Heyl and Larson [1933] have shown that this wax contains the primary alcohols C_{24} and C_{26}. The presence of the C_{31} paraffin has been confirmed by X-ray analysis [Clenshaw and Smedley-Maclean, 1929; Collison and Smedley-Maclean, 1931].

Rose petal wax.

This interesting wax has been investigated by Prophète [1926] who showed that it contained products intermediate in chain length between the glycerides and the common insect and plant waxes. About 50 % of the wax consisted of mixed hydrocarbons, with a melting-point of 53–55° and an iodine number of 8·6. Fractional distillation did not effect any separation between the saturated and unsaturated products, so the mixture (7·04 g.) was brominated in dry ether and the products distilled *in vacuo*. Several fractions were collected and from them by fractional crystallisation products were obtained which were identified as the paraffins C_{16}, C_{20}, C_{21}, C_{22}, C_{23}, C_{26}, C_{27}, C_{30}, together with bromo-derivatives melting at 27° and 38·5–39° respectively. No analyses but only melting-points and boiling-points were quoted. For reasons given earlier in the present paper we do not consider that the identification of these paraffins is valid, for the amount of material in each distillation fraction was small, and on fractional crystallisation the separation of several products from each of them was claimed. Prophète's results however do suggest that paraffins of chain length shorter than C_{25} and possibly olefins are present in petal waxes.

Miscellaneous wax primary alcohols and paraffins.

Paraffin from Cannabis indica (*Hashish*). Dr Cahn kindly placed at our disposal several grams of this material. Fractional crystallisation showed that it was a mixture, and data for the upper and a middle fraction are given in Table VII. C_{29} and C_{31} predominate, and C_{27} is present (*cf.* Cahn, 1931).

Paraffin from corn pollen (Zea Mays). Prof. Anderson kindly placed at our disposal a small amount of this material [*cf.* Anderson, 1923]. By our slow method of taking melting-points the substance melted sharply at 61·2° and set in a very fine crystal mat at 60·8°. It was freely soluble in cold petroleum and was therefore not contaminated with primary alcohol; the melting-point, moreover, was unchanged on treatment with sulphuric acid. It showed no transitions on heating or cooling and gave a poor X-ray photograph (Series B 380). It is undoubtedly a mixture of at least 4 paraffins.

Paraffin from Gymnema Silvestre. Dr E. C. Shorey kindly provided us with a very small sample of this material, which had been isolated by Power and Tutin [1904]. It melted at 67·4–67·6° and set at 67·4° as a whitish mist which deepened in intensity as the temperature fell. No transitions were observed on either heating or cooling, and as the material was freely soluble in cold petroleum we suggest that it was a mixture of at least 4 paraffins.

Paraffin from Myoporum lactum. This was isolated by McDowell [1925], who found that it melted at 62–63° and gave a molecular weight of 470, suggesting C_{32} or C_{34}. Dr McDowell has kindly placed a sample at our disposal for further analysis. The material was freely soluble in cold petroleum, and by our slow method of taking melting-points it melted at 60·7–60·8° and set at 60·3°. No transitions were observed, and the material is a mixture of 3 or 4 paraffins.

Paraffin from supa oil. This was isolated by Henderson *et al.* [1926] and X-ray analysis of a single crystal by Müller [1928] showed that it was C_{29}. No

Paraffin from leaves of cotton plant. This substance, M.P. 75°, was considered by Power and Chesnut [1926] to be C_{35}. We have to thank Dr Chesnut for placing a small sample at our disposal for further identification. The material appeared to become molten around 65–67° and a white mist cleared at 75°. After treatment with sulphuric acid it melted at 61·0–61·6° and showed no transitions on heating or cooling. We suggest that the original material was a mixture of paraffins with a small amount of primary alcohol.

Paraffin from flowers of Tagetes grandiflora. This was isolated by Kuhn *et al.* [1931]. It melted at 68·5–69·5°, had a crystal spacing of 41·7 ± 0·3 Å. and was probably C_{31} with a small amount of higher homologue as they suggest.

Paraffin from American cranberry. From observations based on melting-point, transition temperatures and crystal spacing, Markley and Sando [1934] have shown that this is C_{29} with 10 % to 15 % of C_{31}.

We are greatly indebted to Dr T. A. Henry, Director of the Wellcome Chemical Research Laboratory, for placing at our disposal for further identification the following products isolated by Dr Power and his colleagues many years ago, all of which have been described in published papers.

Paraffin, M.P. 64–65° from *Anthemis nobilis* [Power and Browning, 1914, 1]; M.P. 66° from *Artemisia afra* [Goodson, 1922]; M.P. 65·5° from *Cluytia similis*

[Tutin and Clewer, 1912]; M.P. 65·2° from *Eriodictyon* [Power and Tutin, 1906]; M.P. 65–66° from *Euphorbia pilulifera* [Power and Browning, 1913]; M.P. 63–65° from *Matricaria chamomilla* [Power and Browning, 1914, 2]; M.P. 65·5° from *Solanum angustifolium* [Tutin and Clewer, 1914], all of which were identified as C_{30}. We found that these paraffins melted within 1° of the temperatures stated, but in no case were transitions observed on heating or cooling, showing that each preparation was a mixture of 3 or 4 paraffins and that its identification as C_{30} is no longer justifiable.

Cluytyl alcohol was isolated from *Cluytia similis* by Tutin and Clewer [1912]. The melting-point is given as 82·5°; that of the acetate as 64°, and the alcohol was considered to be $C_{28}H_{58}O$. The data that we obtained (Table VII) show that the substance is a mixture consisting of 30 % C_{26} + 70 % C_{28}.

Incarnatyl alcohol was isolated from *Trifolium incarnatum* by Rogerson [1910]. It is described as crystallising in needles, M.P. 72–74°, to give a benzoate, M.P. 58–60°, and an analysis for carbon and hydrogen suggesting $C_{33}H_{68}O$. We found that the material became molten at 67–68°, giving a dense mist that cleared at about 70°. This suggested to us that it was a mixture of primary alcohol and paraffin, which would account for the high value for carbon obtained on combustion. As only a very small amount of material was available, confirmation was obtained by X-ray analysis, which gave a very diffuse picture similar to that of mixed paraffins. Hummel alcohol and psyllostearyl alcohol have similar low melting-points and high carbon contents, and for these reasons are also considered to be mixtures of alcohol and paraffin.

DISCUSSION.

The comprehensive analysis of the wax products given above shows that in two cases only have the samples of paraffin isolated from natural sources proved to be pure products satisfying the requirements of X-ray analysis, *viz.* C_{29} from supa oil by Müller and C_{31} from spinach leaves by Clenshaw and Smedley-Maclean. In all the cases examined by us they were mixtures and separation of the constituents by fractional crystallisation proved very laborious, so that in one instance only, that from apple cuticle, was it sufficiently complete to give a pure product. But we succeeded in showing that C_{27} and C_{29} are present in this wax, and that C_{31} is present in candelilla wax, and that C_{33}, C_{35} and C_{37} are present in cactus wax. It will be observed that these paraffins include all those with an odd number of carbon atoms from C_{27} to C_{37}.

As stated in the introduction, a review of the literature discloses many claims to the isolation of paraffins with an even number of carbon atoms. Chief among them is C_{30} which has been reported 19 times. Eight of these actual specimens have been placed at our disposal for further analysis, and we state without hesitation that they are mixtures; furthermore we have ourselves isolated from five sources, *Cannabis indica*, wheat, lucerne, perennial ryegrass and runner-bean, products whose melting-points would have suggested to earlier workers that they were this paraffin, and we have been able to show without difficulty that they were mixtures. In addition we have shown that the paraffin of candelilla wax, considered by Meyer and Soyka to be C_{32} is really slightly impure C_{31}, and doubt the claim to the isolation of C_{28} from the oil of *Bombyx mori* as the melting-point quoted (62·5°) is nearly 1° higher than that of this paraffin [Grün and Halden, 1929]. With regard to the samples of paraffin which we have shown to be mixtures, we consider that we have definitely proved the absence of C_{28} from apple cuticle wax, which contains C_{29} and C_{27}; of C_{36} and C_{34} from cactus wax, which contains C_{37}, C_{35} and C_{33}; while in the case of tobacco and *Cannabis indica* paraffins, which were submitted to a long fractionation, no evidence for the presence of even-number carbon members was obtained. The whole of our evidence therefore leads to only one possible conclusion, that all the paraffins present in plant and insect waxes contain an odd number of carbon atoms.

Our chief reasons for assuming that all the primary alcohols and *n*-fatty acids present in waxes contain an even number of carbon atoms were set forth in the introduction, and the relative ease with which we have been able to interpret the composition of the mixtures which occur in waxes by means of melting-point and crystal spacing data based on this assumption does, in our opinion, provide the very strongest collateral proof of it.

For convenience the table summarising the constituents of the various waxes examined is given in the following paper [Chibnall and Piper, 1934]. If we except certain secondary products which are of an extremely limited distribution, we are led to the conclusion that all waxes are of essentially the same general type, and we consider that we have proved the presence in them of even-number primary alcohols and *n*-acids from C_{24} to C_{36}, and of odd-number paraffins from C_{25} to C_{37}. This does not exclude the possibility of the presence of small amounts of still higher or lower even-number fatty acids and alcohols, or odd-number paraffins, but we have as yet no satisfactory proof of their presence.

The waxes differ in chemical composition only in the proportions in which these products are present, and their physical properties are determined not only by the amount and respective chain length of the paraffins, free primary alcohols, free *n*-fatty acids and true wax-esters present, but also by the chain length of the two components of the wax-esters. As examples we may take lac wax, beeswax, and cotton wax. These differ not only in physical properties, but on prolonged fractionation products have been obtained which appeared to previous workers to be different in each particular case. Our analyses show that lac wax consists in large part of free even-number alcohols, from C_{26} to C_{34}, which are soluble in hot ethyl alcohol, and constitute the "lac wax" of commerce, together with wax esters containing the even-number acids from C_{30} to C_{34} and alcohols from C_{30} to C_{36}. The C_{34} acid and alcohol largely predominate and the insolubility of this fraction of the wax suggests that the shorter-chain alcohols are combined in the wax-esters with the longer-chain acids and *vice versa*. Beeswax and cotton wax both contain a series of even-number primary alcohols and acids between C_{26} and C_{34}, yet the relative proportions in which these are present must differ in the two cases, since fractionation under standard conditions has led previous workers to isolate from the former wax a series of alcohols and acids that appeared to contain an odd number of carbon atoms, and from the latter a series that appeared to contain an even number of carbon atoms. Furthermore, both contain the C_{34} alcohol and acid, yet as neither yields an ethyl alcohol-insoluble wax corresponding to lac wax it seems that the ester of these two products cannot occur, but that both of them must be coupled with shorter-chain (C_{26}, C_{28}) products, thus making the respective esters more soluble.

The present work makes it abundantly clear that all the names which have been assigned by previous workers to primary alcohols, fatty acids and paraffins isolated from waxes have been given to products that are not chemical entities but mixtures. In a previous paper [Pollard *et al.*, 1931] we discussed the term "ceryl" alcohol and recommended that it be retained for mixed alcohols, M.P. *circa* 80° isolated from natural sources. We now withdraw that recommendation, as we do not feel justified in making a similar one in connection with the names "myricyl" alcohol, "melissyl" alcohol, "melissic" acid and "cerotic" acid, all of which have been current in the literature for very many years. Chemists have rightly ceased to make use of names given to mixtures of glyceride fatty acids by earlier workers, and we consider that the same should apply to mixtures in the wax series. To continue to refer to an acid of melting-point 89–90° isolated from natural sources as "melissic" acid, with the implied meaning that it is either C_{30} or C_{31}, when we know that the C_{30} acid melts at 94°, the C_{31} at 93°, and that the naturally occurring acid is a mixture of (probably) $C_{30}+C_{32}+C_{34}$ is contrary to progress. Such naturally occurring acids (or alcohols) should be designated simply as acids (or alcohols) of M.P. 89–90°, the chemical name sufficing for those which can be shown to be pure substances. We therefore recommend that the following names be abandoned, for we have shown in the present series of papers dealing with wax constituents that they have always been applied to products that are mixtures: ceryl alcohol, montanyl alcohol, myricyl alcohol, melissyl alcohol, medicagol, cluytyl alcohol, incarnatyl alcohol, psyllostearyl alcohol, laccerol, tachardiacerol, neoceryl alcohol, hummel alcohol, gossypyl alcohol, pesang alcohol, raphia alcohol, cerotic acid, montanic acid, melissic acid, lacceroic acid, tachardiacerinic acid, neocerotic acid, ghedda acid, gossypic acid, carnaubic acid, alfalfone, tachardiacerin. We also recommend that the following names, applied to substances that are clearly mixtures, but whose composition we cannot suggest because no derivatives have been prepared, be also abandoned. Loranthyl alcohol, M.P. 71–72° [Zellner *et al.*, 1924]; tarchonyl alcohol, M.P. 82° [Canzoneri and Spica, 1882]; mesembrol, M.P. 73–74° [Hartwick and Zwicky, 1914]; and the paraffins bryonan [Etard, 1892]; lauran [Matthes and Sander, 1908], and petrosilan [Matthes and Heintz, 1909], all three of which melt at 69° and were considered to be C_{20}. They are presumably impure C_{31}.

SUMMARY.

Comprehensive analyses have been made of the alcohols, fatty acids and paraffins present in numerous plant and insect waxes.

The alcohols are mixtures of even-number alcohols from C_{24} to C_{36}. Occasionally in certain leaf waxes one particular alcohol is present to the virtual exclusion of all others, and isolation in pure form is possible.

The acids are mixtures of even-number *n*-fatty acids from C_{24} to C_{34}, and isolation of pure acids has not been thought possible or attempted.

The paraffins consist of one or more of the odd numbers from C_{25} to C_{37}. Good evidence has been obtained that even-number paraffins do not occur.

By making use of the melting-point and crystal spacing data for mixtures of pure primary alcohols, *n*-fatty acids and paraffins it has been found possible to suggest the composition of the mixed products found in waxes.

As numerous alcohols, acids and paraffins isolated by previous workers are shown to be mixtures and not chemical entities it is recommended that the names used to designate these products be abandoned.

In conclusion we should like to thank Dr J. A. B. Smith and Dr R. C. Jordan for help in certain of the analyses; Mr A. J. Gibson, Special Officer Lac Inquiry, India House, and Mr T. Hedley Barry for samples of stick lac and commercial lac wax, and all those specifically mentioned by name in the text who have kindly provided us with samples of wax products for purposes of identification.

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