

# CCXXVI. THE WAX CONSTITUENTS OF THE APPLE CUTICLE.

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## INTRODUCTION.

IN the introduction to the preceding paper dealing with the synthesis and crystal spacings of certain higher paraffins, alcohols and ketones, it was pointed out that the object of the work was to enable a more thorough investigation of the chemical constitution of various plant waxes, especially leaf waxes, to be made than had hitherto been possible. The leaf waxes to which it was hoped particular attention could be given were those derived from various forage grasses, and preliminary investigations showed that these differed from that found by Channon and Chibnall [1929] in the cabbage leaf in that they contained large amounts of alcohols instead of a paraffin and ketone and were therefore not immediately amenable to the separation used by these authors. As a preliminary to work on the grasses it was necessary to devote some time to experiments, the aim of which was to separate fairly completely the following classes of compounds, *viz.* paraffins, ketones, primary alcohols and secondary alcohols, and because a survey of the literature suggested that these might all occur in the wax obtained from apple peel, that material, which can readily be obtained in quantity, was used.

Before describing the actual scheme of separation it may perhaps be as well to state briefly the results of previous workers who have investigated this apple peel wax. Power and Chesnut [1920] isolated a substance, m.p. 63°, which appeared to be a hydrocarbon and which was identified from its melting-point as *n*-triacontane C<sub>30</sub>H<sub>62</sub>. A more comprehensive investigation was made by Sando [1923], who extracted the peel with ether and obtained two groups of substances, one insoluble in light petroleum with which we are not now concerned, and the other soluble in light petroleum, from which he was able to obtain, by intensive fractional crystallisation, the hydrocarbon previously identified by Power and Chesnut and an alcohol melting at 81·5 to 82°. This alcohol gave an acetate melting at 44–46° and was therefore thought to be different from ceryl alcohol, which gives an acetate, m.p. 64–65°. 14-Heptacosanol, previously synthesised by Kipping [1893] melted at 80·5–81·5°

and gave an acetate melting at 45–45.5°. On a similarity of melting-point and without any further evidence at all, Sando identified his apple alcohol with this secondary alcohol, a finding which has been accepted without question by Rivière and Pichard [1924]. Sando's results suggested that other products besides this alcohol and hydrocarbon were probably present in his apple material. Previous work carried out by Power and also in this laboratory suggested that these other products might be primary alcohols or ketones, and the wax, therefore, seemed to be an excellent raw material with which to experiment on methods to separate these four products.

#### A METHOD FOR THE SEPARATION OF THE COMPONENTS OF PLANT WAXES.

It has long been known that primary alcohols, such as ceryl and myricyl alcohol, form hydrogen phthalates which give sodium salts insoluble in water, and this property has been utilised by a number of workers, notably by Power and his colleagues, for the isolation of such alcohols from plant extracts and especially from essential oils. With a view to finding out the applicability of this reaction to the separation of primary alcohols from material that may contain paraffins, ketones and perhaps secondary alcohols, experiments were made to find out the optimum conditions for the conversion of the alcohol into its hydrogen phthalate.

Using ceryl alcohol (m.p. 79.7°) obtained from Chinese wax it was found that heating with an excess of phthalic anhydride with or without pyridine at 120° for 18 hours gave the half ester in almost theoretical yield. On shaking the ethereal solution with aqueous sodium carbonate the insoluble sodium salt was precipitated and could be separated by centrifuging. The alcohol could be recovered from this sodium salt or from the half ester in good yield by saponification with alkali. When a secondary alcohol, however, such as 14-heptacosanol, was submitted to similar treatment it was found that the hydrogen phthalate when shaken in ethereal solution with aqueous sodium carbonate gave no precipitate of insoluble sodium salt. The sodium salt, in fact, was found to be extremely soluble in both ether and alcohol and insoluble in water. This at once suggested a possible method of separating a secondary carbinol not only from primary alcohols but also from paraffins and ketones, and in practice it has been found to work extremely well.

The crude plant wax is first saponified to break down any true wax esters and the unsaponifiable material removed in the usual way. This unsaponifiable material is then heated for 18 hours with phthalic anhydride to convert all the alcohols present into hydrogen phthalates. Excess of phthalic anhydride is then removed and the mixed phthalates, together with any unchanged material, dissolved in ether. The ethereal solution is then gently shaken with a slightly warm aqueous solution of sodium carbonate. The sodium salts of the primary phthalates are precipitated and can be quantitatively separated by appropriate treatment. The ethereal solution which now contains the sodium salts of the secondary phthalates and perhaps ketones and paraffins is evaporated

to dryness and taken up in a relatively small volume of boiling alcohol. On cooling, the paraffins and ketones crystallise out quantitatively leaving in the mother-liquor the very soluble sodium salts of the secondary alcohol esters. The mixture of paraffins and ketones can then be separated by the method used by Channon and Chibnall [1929] in which the ketone is converted into its oxime and advantage is taken of the greater solubility of this oxime in solvents such as light petroleum and mixed ether-acetone. It will be seen that this relatively simple procedure separates at once the primary alcohols, the secondary alcohols, ketones and paraffins into four distinct fractions, each of which can be readily purified. In practice certain modifications have been found essential. For instance, a second treatment with phthalic anhydride for 18 hours is always necessary to ensure that all the alcohols have been converted into their hydrogen phthalates. Also recent experience has shown that it is advantageous to remove hydrocarbons—which are fairly readily soluble in cold light petroleum—before treatment with phthalic anhydride. The method and its modifications will be dealt with in greater detail in the practical part of this and succeeding papers.

#### THE METHOD OF SEPARATION APPLIED TO APPLE PEEL WAX.

Applied to the unsaponifiable portion of apple peel waxes (50 g.) this method of separation yielded without any difficulty a secondary alcohol, a mixture of primary alcohols and a mixture of paraffins but no ketones.

*The secondary alcohol.* The alcohol recovered after saponification of the ether- and alcohol-soluble sodium salts on purification melted at 81.9–82.2° and was obviously the product previously isolated by Sando [1923], for it gave an acetate melting at 44.5–45°. A mixed melt however with 14-heptacosanol was depressed to 70–71°, showing conclusively that Sando's guess was wrong. One of the reasons which prompted us initially to investigate apple peel wax was the belief that this alcohol might be 15-nonacosanol, the secondary alcohol corresponding with the ketone, 15-nonacosanone and the paraffin, *n*-nonacosane, isolated by Channon and Chibnall [1929] from the cabbage. Synthetic 15-nonacosanol, however, melted somewhat higher, 83.8–84°, and a mixed melt was depressed to 78.5–79.5°, showing that the alcohols were not identical. The carbinol had a spacing of 38.65 Å., indicating a straight chain of 29 carbon atoms. Furthermore, the intensity of the different orders of reflection showed that the polar group must be about one-third of the distance along the chain from one end because the third and sixth orders were missing. X-ray analysis, therefore, suggested that the alcohol must be either 9-nonacosanol or 10-nonacosanol. To verify this supposition it was necessary to synthesise both these alcohols *via* the corresponding ketones. Meanwhile, in order to meet the synthesis, the apple alcohol was oxidised by means of chromium trioxide in glacial acetic acid to the corresponding ketone. The apple ketone, m.p. 74.7–74.9°, showed no depression when mixed with synthetic 10-nonacosanone, m.p. 74.5–75°, and a depression of 5° when mixed with synthetic 9-nonacos-

none, m.p. 73.6–74.3°. Furthermore, the crystal spacings (Series Nos. C 35, C 49) of the derived apple ketone and of the synthetic 10-nonacosanone were identical (38.9 Å.), both, as before, having the third and sixth orders missing. The identity of the ketone derived from the apple alcohol was therefore definitely established. On reducing the synthetic 10-nonacosanone to the corresponding carbinol, 10-nonacosanol, however, it was found to melt at 75–75.2°, *i.e.* 7° lower than the apple alcohol. A mixed m.p. (78–79°) was about midway between the melting-points of the two substances, whereas a mixed melt of the apple alcohol with synthetic 9-nonacosanol (m.p. 75.5–75.8°) was depressed to 70°. Clearly the apple alcohol was not identical with the synthetic 10-nonacosanol, although no doubt closely allied to it. This finding at once drew our attention to the fact that if the apple alcohol had the constitution which its derived ketone suggested, it would possess an asymmetric carbon atom and therefore would be optically active, whereas the alcohol obtained by reduction of the ketone would of course be inactive. Dr Hirst of the University of Birmingham kindly undertook to observe the rotation of the apple alcohol, and he reported as follows: "The substance was examined in solution in chloroform (*c*, 1 %). The rotation observed in a 4 dm. tube was certainly less than the experimental error due to the instrument ( $\pm 0.01^\circ$ ). Photographic examination over the range  $\lambda$  7000– $\lambda$  3800 in a 2 dm. tube showed that throughout this range there was no observable rotation (limits of experimental error here were  $\pm 0.02^\circ$ ). It is therefore most improbable that the substance is optically active either in chloroform or in any other solvent." There remained the possibility that the length of the two paraffin side-chains was so great that optical activity might be reduced below measurable limits. This hypothesis was confirmed when the ketone obtained from the apple alcohol by oxidation was reduced. The regenerated alcohol melted at 74.5–74.9° and gave no depression in m.p. with the synthetic product, which melted at the same temperature. Attempts to racemise the natural product by heating gave no change in melting-point. At a later stage of the research we were able to prepare some 4 g. of the hydrogen phthalate. A 20 % solution in chloroform gave a small but definite rotation  $[\alpha]_{5461}^{17.5} = +0.62^\circ$ . The apple secondary alcohol is therefore undoubtedly *d*-10-nonacosanol and it belongs to a small group of optically active compounds, of which mannitol is a well known example, exhibiting no appreciable rotation. It is possibly identical with the alcohol, m.p. 82.5°, isolated by Kawamura [1928] from the fruit of *Ginkgo biloba* which gave an acetate m.p. 43–43.5° and a ketone m.p. 74°.

*Primary alcohols.* The ether-insoluble sodium salts of the primary phthalates were saponified and yielded, after purification by crystallisation, 4.1 g. of primary carbinol. This material melted at 81° and when submitted to an intensive fractional crystallisation yielded fractions differing in m.p. by 1 and 2°. Investigations of these fractions soon convinced us that we were dealing with a mixture of primary alcohols of the type usually referred to as ceryl alcohol, the constitution of which will be discussed in greater detail in the

following paper. An upper fraction, m.p. 81.7–82.2°, gave an analysis corresponding closely with *n*-octacosanol. (Found: C, 81.8; H, 14.1.  $C_{28}H_{58}O$  requires C, 81.8; H, 14.2 %.) The crystal spacing (Series No. C. 56) was 77.6 Å. which suggests that the product, according to the data of Malkin [1930] was  $C_{29}H_{60}O$ . On reduction *via* the iodide it gave a paraffin m.p. 62.2–62.8°. The crystal spacing (Series No. C. 72) was 38.4 Å. with only 5 orders showing, and the paraffin was clearly a mixture. A lower fraction, m.p. 80.2–80.7°, gave a spacing (Series No. C. 33) of 76.1 Å. and a derived paraffin which melted at 60–60.2°. The crystal spacing (Series No. C. 46) of this was 37.4 Å. with only three orders showing and again the material was definitely a mixture. All the fractions of this primary alcohol therefore were united and reduced *via* the iodide to the corresponding paraffin, m.p. 61.0–61.5°, which was submitted to an intense fractionation by means of light petroleum. This was found to be very laborious. After about 100 fractions had been dealt with the upper and lower limits of m.p. were 62.8° and 59.5°, but the main bulk of material still melted around 61° and 62°. The highest and lowest fractions were again treated with sulphuric acid. At this stage of the resolution we found that all the fractions showed certain characteristics. (1) The m.p. was sharp, and about 0.2–0.3° above the s.p. (2) On setting, the molten material crystallised out in fine needles. (3) The transition changes were well marked, but the temperature range in some cases was 1–2°. This convinced us that we were dealing with a mixture containing not more than 3 components in recognisable amounts, but the resolution was not sufficiently complete for X-ray analysis to be of any use. A fraction (Series No. B. 80) of m.p. 61.4–61.6°, corresponding to  $C_{28}H_{58}$ , gave a spacing 38.3 Å. with a fair number of orders, a melted layer however gave a poor picture and a spacing of 37.8 Å. This distortion of a spacing on melting is characteristic of a mixture of at least three paraffins, and showed that it was necessary to carry the fractionation still further. After another 150 fractions had been dealt with we reached a stage when further fractionation by this means seemed impossible. We had then 22 samples. All the fractions set on cooling in large crystal masses, but in none was the transition-temperature as high as that of a corresponding pure paraffin. We drew the conclusion that the fractionation had separated the three components to such an extent that each sample now contained only two of these in recognisable amounts. Ten representative samples were then X-rayed, and as was expected, the picture obtained was sufficiently good to enable a comprehensive analysis of the mixtures to be obtained.

A review of the data in Table I shows that it is possible to account for all the melting- and transition-points and the spacings from suitably chosen mixtures of  $C_{30}H_{62}$ ,  $C_{28}H_{58}$  and  $C_{26}H_{54}$ . On this assumption the bulk of the material in B. 115 is the middle paraffin with about equal amounts of the higher- and lower-melting constituents as impurities. In B. 110–B. 114 we have mainly  $C_{30}H_{62}$ ,  $C_{28}H_{58}$ ; in B. 116–B. 119  $C_{28}H_{58}$ ,  $C_{26}H_{54}$ . The transition-temperatures are nearly all slightly lower than those of the suggested binary

Table I. Paraffin fractions obtained from the mixed primary alcohols.

Series No.	Transition-point on heating		Transition-point on Spacing		M.P.	S.P.	Transition-point on heating	Remarks
	Series No.	Spacing A.	Series No.	Spacing A.				
B. 110	63.2-63.6°	63.2°	39.25	39.25	63.2°	—	54.8	The photograph of B. 110 is definitely that of a paraffin with 30-40% contamination. C. 97 gives very good pictures.
	Computed data.	63.6°	C. 97	39.15	63.6°	63.1°	57.2°	
B. 112	63.1-63.3°	63.1°	38.85	38.85	63°	—	55.2°	A very small % of C <sub>29</sub> H <sub>60</sub> or C <sub>27</sub> H <sub>54</sub> in the C <sub>29</sub> H <sub>60</sub> would give the correct spacing. The photograph however is definitely that of a paraffin with 30-40% of contamination.
	Computed data.	63.3°	Computed data.	39.25	63.3°	—	55°	
B. 113	62.6-62.8°	62.4°	38.55	38.55	62.5°	—	54.8°	
	Computed data.	62.8°	B. 89	38.5	62.5°	62.3°	55.6°	
B. 114	62.2-62.6°	62°	38.55	38.55	62°	—	54°	
	Computed data.	62.6°	B. 89	38.5	62.3-62.6°	62.3°	50-52°	
B. 125	62.3-62.5°	62.3°	38.5	38.5	62.3-62.5°	62.3°	53-53.5°	
	Computed data.	62.5°	B. 125	38.5	62.3-62.5°	62.3°	58-58.5°	

Synthetic mixture

Series No.	Composition
Computed data.	{ 60% C <sub>29</sub> H <sub>58</sub> 40% C <sub>27</sub> H <sub>54</sub>
C. 97	{ 95% C <sub>29</sub> H <sub>60</sub> 5% C <sub>27</sub> H <sub>54</sub>
Computed data.	{ 90% C <sub>29</sub> H <sub>60</sub> 10% C <sub>27</sub> H <sub>54</sub>
Computed data.	{ 65% C <sub>29</sub> H <sub>58</sub> 35% C <sub>27</sub> H <sub>54</sub>
Computed data.	{ 65% C <sub>29</sub> H <sub>58</sub> 35% C <sub>29</sub> H <sub>60</sub>
Computed data.	{ 75% C <sub>29</sub> H <sub>58</sub> 25% C <sub>27</sub> H <sub>54</sub>
B. 89	{ 50% C <sub>29</sub> H <sub>60</sub> 50% C <sub>27</sub> H <sub>58</sub>
Computed data.	{ 35% C <sub>27</sub> H <sub>58</sub> 65% C <sub>29</sub> H <sub>60</sub>
B. 122	{ 34% C <sub>29</sub> H <sub>54</sub> 66% C <sub>29</sub> H <sub>60</sub>
B. 89	{ 50% C <sub>29</sub> H <sub>60</sub> 50% C <sub>27</sub> H <sub>58</sub>
Computed data.	{ 75% C <sub>29</sub> H <sub>58</sub> 25% C <sub>27</sub> H <sub>58</sub>
B. 125	{ 74% C <sub>29</sub> H <sub>58</sub> 24% C <sub>27</sub> H <sub>58</sub> 2% C <sub>29</sub> H <sub>54</sub>

Series No.	M.P.	S.P.	Transition-point on heating	Spacing A.	Synthetic mixture		Transition-point on heating	Spacing A.	Remarks		
					Series No.	Composition					
B. 115	61.2-61.5°	61.2°	52-52.7°	38	Computed data	$\left\{ \begin{array}{l} 85\% \text{ C}_{37}\text{H}_{58} \\ 15\% \text{ C}_{38}\text{H}_{62} \end{array} \right.$ C. 114	62.1°	—	54.8°	38	
B. 123						$\left\{ \begin{array}{l} 50\% \text{ C}_{37}\text{H}_{56} \\ 50\% \text{ C}_{38}\text{H}_{60} \end{array} \right.$	61.4-61.5°	61.1°	57-57.4°	37	There was no appearance of the shorter spacing in B. 115.
B. 70						$\left\{ \begin{array}{l} 90\% \text{ C}_{38}\text{H}_{58} \\ 5\% \text{ C}_{36}\text{H}_{54} \\ 5\% \text{ C}_{39}\text{H}_{62} \end{array} \right.$	61.1-61.3°	60.9°	52-52.5°	38.2	
B. 69						$\left\{ \begin{array}{l} 90\% \text{ C}_{38}\text{H}_{58} \\ 5\% \text{ C}_{37}\text{H}_{56} \\ 5\% \text{ C}_{39}\text{H}_{60} \end{array} \right.$	61.3-61.5°	61.2°	54-54.4°	38	Appears to be the most probable constitution.
B. 116	59.9-60.1°	59.8°	50-51°	37.65	B. 47	$\left\{ \begin{array}{l} 70\% \text{ C}_{38}\text{H}_{58} \\ 30\% \text{ C}_{36}\text{H}_{54} \end{array} \right.$	60.1-60.2°	60°	52°	37.7	
B. 117	58.0-58.4°	58°	47.2-47.4°	36.65	B. 46	$\left\{ \begin{array}{l} 60\% \text{ C}_{38}\text{H}_{54} \\ 40\% \text{ C}_{37}\text{H}_{56} \end{array} \right.$	58.6°	58.2°	49.5°	36.8	
B. 118	57.2-57.6°	57.1°	46-46.5°	36.24	B. 44	$\left\{ \begin{array}{l} 90\% \text{ C}_{37}\text{H}_{54} \\ 10\% \text{ C}_{38}\text{H}_{56} \end{array} \right.$	59.2-59.5°	58.9°	52.5-53°	36.6	
B. 119	56.8-57°	56.6°	46.5-47°	35.55	B. 42	$\left\{ \begin{array}{l} 70\% \text{ C}_{38}\text{H}_{54} \\ 30\% \text{ C}_{37}\text{H}_{58} \end{array} \right.$	58°	57.5°	49.5°	36.2	
					Computed data	$\left\{ \begin{array}{l} 90\% \text{ C}_{38}\text{H}_{54} \\ 10\% \text{ C}_{37}\text{H}_{56} \end{array} \right.$	57°	56.3°	49.5°	35.25	
					B. 124	$\left\{ \begin{array}{l} 55\% \text{ C}_{38}\text{H}_{54} \\ 45\% \text{ C}_{37}\text{H}_{56} \\ 88\% \text{ C}_{38}\text{H}_{54} \\ 9\% \text{ C}_{39}\text{H}_{58} \\ 3\% \text{ C}_{37}\text{H}_{56} \end{array} \right.$	57.7°	—	49.8°	35.6	
							56.8-57°	56.6°	47°	35.51	

The most probable constitution of each fraction is given in heavy type.

mixture, probably owing to the presence of traces of the lower paraffin in the top, and the higher in the lower fractions. We have verified this by making appropriate mixtures containing small amounts of the suggested impurity, as shown in the case of B. 114 and B. 119. There is no change in the m.p., s.p. or crystal spacing, but the transition-point is depressed about  $2^\circ$ . We have in each case tested the possibility of "odd + even" and "2 odd" paraffin combinations. While in some cases agreement is fair, in others it is necessary to assume only small amounts of one component, and the X-ray pictures definitely reject such a mixture. The behaviour of the material after partial fractionation, as mentioned above, suggests that it does not contain more than three components in any appreciable amount, and the three even number paraffins suggested above are the only ones that give consistent results throughout the series. Observations on the second transition-points prove that the chains do not differ by three or more carbon atoms, also if any one of the fractions is in the main composed of paraffins differing by four carbon atoms, the photographs would have shown the two sets of spacings. We consider that the above results show conclusively that the mixed primary alcohol consisted of *n*-triacontanol, *n*-octacosanol and *n*-hexacosanol.

*The paraffin fraction.* The crude fraction, recrystallised from carbon disulphide, melted at  $63\text{--}64^\circ$ . The product was slightly brown and was therefore dissolved in boiling alcohol and clarified with charcoal. The resulting product melted at  $63.2\text{--}63.6^\circ$ , and set at  $62.4^\circ$  with no definite crystal form. The material, in fact, reminded us of the crude hydrocarbon fraction of cabbage from which the ketone had not yet been separated. A portion of the product was therefore oximated and an attempt made to separate a ketoxime soluble in light petroleum, but none was found. Furthermore analysis showed that no large amount of a ketone or other oxygen-containing substance could be present in the mixture. The material was therefore repeatedly treated with sulphuric acid at  $130^\circ$  until no further blackening occurred. Contrary to our expectations the m.p. was not raised but was lowered to  $62.6\text{--}63^\circ$ . The crystal spacing was  $38.7 \text{ \AA}$ . (Series No. C. 29). This spacing at the time seemed quite correct for  $\text{C}_{29}\text{H}_{60}$ . The m.p., moreover, agreed with that of the same material isolated by Channon and Chibnall [1929] from cabbage. But synthetic  $\text{C}_{29}\text{H}_{60}$  melted at  $63.4\text{--}63.6^\circ$  and no amount of purification with sulphuric acid enabled us to raise the m.p. of the apple product above  $63^\circ$ . We were therefore forced to the conclusion that this apple product must be a mixture and in spite of this was giving a correct crystal spacing for a pure paraffin. This led us to the investigation of the crystal spacing of mixed paraffins and we then found that the spacing, for example  $38.7 \text{ \AA}$ ., of a pure paraffin could also be the spacing given by a mixture, as has been shown in the preceding paper [1931] dealing with these paraffins and their mixtures. We therefore attempted to fractionate the product derived from the apple and found that this could be slowly but effectively accomplished by making use of the different solubilities of the paraffins in cold petroleum (b.p.  $40\text{--}60^\circ$ ). The fractionation, practical

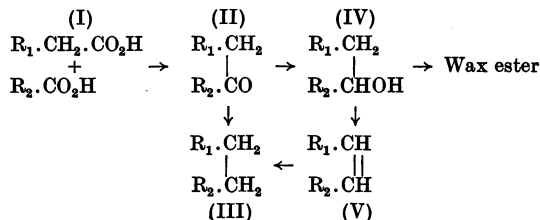


details of which are given later, finally gave us a series of products whose melting-points and crystal spacings are given in Table II. The highest melting fraction, 63.4–63.6°, was further fractionated by means of petroleum (B.P. 40–60°) into three samples (C. 133–135). From the discussion which we gave in the preceding paper on the criteria necessary for judging the purity of a paraffin we consider that their melting- and transition-points and crystal spacings are definite proof that a highly pure sample of  $C_{29}H_{60}$  has been isolated. When the data given in Table II are viewed as a whole it is seen that the variation in the crystal spacing with the melting-point is very similar to that which would be given by a mixture of  $C_{29}H_{60}$  and  $C_{27}H_{56}$ . In the sixth column of Table II we have given the percentage composition calculated from the m.p. of a mixture of  $C_{29}H_{60}$  and  $C_{27}H_{56}$  and in column 7 the crystal spacing of such a mixture calculated from the synthetic mixture of  $C_{31}H_{64}$  and  $C_{29}H_{60}$  given in the previous reference. It will be observed that the suggested and observed crystal spacings are in very close agreement. We would call particular attention to fraction C. 138. Its melting-point suggests that it could be either  $C_{28}H_{58}$ , equimolar  $C_{27}H_{56} + C_{29}H_{60}$  or 66 %  $C_{29}H_{60} + 34$  %  $C_{26}H_{54}$ . A comparison of the crystal spacings and the transition-points shows clearly that it is equimolar  $C_{27}H_{56} + C_{29}H_{60}$ . Moreover we did not find, during the course of the fractionation, any product melting between 61.4–61.6° which could not be readily separated into a series of fractions having m.p. both higher and lower. We therefore draw the definite conclusion that the mixed paraffin contains only  $C_{29}H_{60}$  and  $C_{27}H_{56}$  and that  $C_{28}H_{58}$  and  $C_{26}H_{54}$  are absent.

## GENERAL DISCUSSION.

Summarising the above results it will be seen that we have been able to prove definitely the presence in the apple peel wax of the following substances, *n*-nonacosane, *n*-heptacosane, *d*-10-nonacosanol, *n*-triacontanol, *n*-octacosanol and *n*-hexacosanol.

It is interesting to consider these results in relation to the hypothesis advanced by Channon and Chibnall [1929] on the metabolism of paraffins in the plant. If the paraffin (III) is synthesised from the fatty acids (I) *via* the ketone (II), then the discovery of a secondary alcohol such as *d*-10-nona-



cosanol together with *n*-nonacosane in the same wax is interesting. Both could, of course, be formed by reduction of the corresponding ketone, yet the reduction of —CHOH— to —CH<sub>2</sub>— is so difficult, both chemically and by the aid of living organisms, that one cannot suggest that the alcohol is an

Table II. *Melting-points and crystal spacings of the paraffin fractions (with certain standards for comparison).*

Series No.	Transition-point on heating	Melting-point	Setting-point	Transition-point on cooling	Suggested composition %		Crystal spacing	
					C <sub>27</sub> H <sub>56</sub>	C <sub>28</sub> H <sub>60</sub>	Observed A.	Calculated from Col. 6 A.
C. 133	57.2-57.5°	63.4-63.6°	63.2°	55.8°	0	100	38.8	38.7
C. 134	57.5-57.8°	63.4-63.6°	63.2°	56.0°	0	100	38.6	38.7
C. 135	57-57.3°	63.4-63.6°	63.2°	55.3°	0	100	38.75	38.7
C. 136	55.2-55.8°	62.7-62.9°	62.3°	54.7°	17	83	38.85	38.8
C. 137	53.5-54°	61.5-61.8°	61.4°	52°	40	60	38.65	38.5
C. 138	52-52.5°	61.4-61.6°	61.2°	51.2°	44	56	38.35	38.4
C. 139	52°	60.5-60.7°	59.6°	50.4°	62	38	38.0	38.1
C. 140	51°	59.8-60.2°	58.8°	48.5°	76	24	37.4	37.4
C. 26	57.5°	63.4-63.6°	63.2°	56.0°	Composition of standards		36.68	
C. 114	57.2-57.4°	61.2-61.5°	61.1°	53.7°	C <sub>28</sub> H <sub>60</sub>		37.0	
C. 22	52.8-53°	59.0-59.1°	56.5°	51°	C <sub>27</sub> H <sub>56</sub>		33.25	
B. 122	50-52°	60.7-61°	60.7°	Indistinct	66% C <sub>27</sub> H <sub>56</sub>		36.4	
B. 123	52-52.5°	61.1-61.3°	60.9°	51-50.5°	34% C <sub>28</sub> H <sub>60</sub>		38.6	
					Equimolar			
					C <sub>27</sub> H <sub>56</sub> + C <sub>28</sub> H <sub>60</sub>		38.2	

intermediary product in the direct reduction of the ketone to the paraffin. If the alcohol and paraffin have a common origin, and the fact that they both contain 29 carbon atoms is strong evidence on this point, then one would suggest (1) that the secondary alcohol had been formed by partial reduction of the ketone and that it is an alternative end-product to the hydrocarbon. It might, of course, unite with a long-chain fatty acid to form a true wax. (2) That the ketone (II) undergoes a reduction first to the secondary alcohol (IV) and then to the corresponding paraffin (III) *via* the corresponding olefine (V). Such a reduction involving the loss of water and the creation of a double bond is well known in biological reactions (*cf.* the interconversion of malic and succinic acids *via* the unsaturated fumaric acid). It is true that in the present case the ketone has not been found, but support for the hypothesis that it is the precursor of both the secondary alcohol and paraffin is obtained from a later research, in which it is shown that 15-nonacosanol, 15-nonacosanone and *n*-nonacosane are all present in the wax of the Brussels Sprout leaf. Our own researches have not yet given any definite evidence for the presence of olefines in plant waxes. There are, however, a few references in the literature to the isolation of products that appear to contain only carbon and hydrogen in the proportion close to that found in the paraffins and which have been stated to possess small iodine values. It is possible therefore that olefines corresponding with these ketones, alcohols and paraffins are present in the plant waxes.

Channon and Chibnall [1929] when they first put forward the hypothesis that the ketone might arise through the condensation of two molecules of fatty acids drew attention to the fact that the ketone they found in the cabbage leaf, 15-nonacosanone would postulate the presence in the plant at some stage in the synthesis of pentadecic acid, and because the presence of this acid in natural products has not been clearly demonstrated, they considered that the hypothesis should be accepted with great caution. In the present instance the two fatty acids required to give the postulated intermediate ketone would be decic and eicosanic acids, both of which have been found in plants, although neither of them can be considered a commonly occurring fatty acid. It will be seen then that the discovery of this secondary alcohol in the apple wax does, to a certain extent, support the original contention that these long-chain products can arise from the shorter fatty acids.

We still feel however that caution is necessary. We believe that for the first time some insight is being gained into the constitution of the mixed primary alcohols which we ourselves invariably find in these plant waxes. We cannot lose sight of the possibility that these primary alcohols are as closely connected with the paraffins as are the secondary products. If so, then we should expect to find some general relationships between the number of carbon atoms in the paraffins and in the primary alcohols. We have as yet very few data concerning this point, and further discussion at the present time does not seem profitable. Our chief difficulty in collecting such data lies in the fact that those waxes which contain a large proportion of prim alcohol

(*e.g.* Chinese wax, raphia wax) do not contain paraffins or secondary products, and those which contain paraffins (*e.g.* Candalilla wax) have only a small amount of primary alcohols. From the point of view of paraffin metabolism it is essential that all these be found in one and the same wax, and it is for that reason that we have thought it worth while to resolve, with much labour, the very small amount of mixed primary alcohol (5 g.) which we isolated from the apple peel.

#### EXPERIMENTAL.

*Extraction of the wax.* Messrs Lyons kindly provided us with a large quantity of apple peel which had been dried *in vacuo* at 40°. It was powdered in a coffee mill and then extracted with petroleum (B.P. 40–60°) in a Soxhlet for 15 hours. The extract from 8 kg. of peel was concentrated to 500 cc. and treated with two volumes of acetone. The precipitated wax was filtered off, again dissolved in the same volume of petroleum and precipitated as before. Dried *in vacuo* the crude wax (57 g.) was a yellow-brown, brittle solid of no definite m.p.

*Saponification of the wax.* An alcoholic solution of potassium hydroxide (170 cc. of 4.5 %) was added to the wax (57 g.) dissolved in warm benzene (300 cc.) and the mixture boiled for 3 hours on the water-bath. Water (300 cc.) was added and the mixture again heated for 10 minutes. The benzene layer was then separated, the aqueous solution extracted with benzene and the benzene solutions combined. On removal of the solvent by distillation the unsaponifiable matter was obtained as a pale brown hard wax (50 g.) m.p. 63.5°.

*Treatment with phthalic anhydride.* The unsaponifiable material (50 g.) was heated with phthalic anhydride (50 g.) and pyridine (20 cc.) for 18 hours at 120° in an oil-bath. In order to remove the pyridine the hot molten mixture was then poured into dilute hydrochloric acid and stirred while the mixture was heated to boiling-point. After cooling, the solid layer of phthalates was separated and heated with successive quantities of water to remove the residual phthalic anhydride. The mixture was allowed to cool and the solid cake of crude esters was collected and dissolved in warm ether (1500 cc.), giving a light brown solution. This was cautiously agitated with an excess of slightly warm aqueous solution of sodium carbonate, which caused the separation in the aqueous phase of the insoluble white sodium salts of the primary phthalic esters. The lower layer, which was an emulsion of water, ether and suspended solid matter, was separated from the supernatant ether and centrifuged. By this means the solid sodium salts collected at the ether-water interface and could be readily removed. This semi-solid mass was freed from most of the ether-soluble matter with which it was contaminated by shaking with a large volume of ether and centrifuging the resultant emulsion. The solid was then dried *in vacuo* and repeatedly extracted with dry ether. There remained a grey powder (12 g.) of no definite m.p. Many experiments on the separation of these primary phthalates have shown that the precipitated sodium salts are more granular and less gelatinous if the sodium carbonate solution is slightly warm.

Even so they are difficult to filter and can only be effectively separated by centrifuging.

After separation of these insoluble sodium salts the ether solutions were combined and the solvent removed. The residue was taken up in boiling alcohol and on cooling an impure paraffin fraction (32.2 g.) crystallised out. The mother-liquor on removal of the solvent yielded 7.2 g. of a gum which was readily soluble in cold ether, alcohol and most organic solvents, and which was the sodium salt of the secondary alcohol phthalate. The crude paraffin fraction (32.2 g.) was again heated with phthalic anhydride (15 g.)—this time without pyridine—for 18 hours at 120° and then treated as before. On shaking the ethereal solution with aqueous sodium carbonate, a slight precipitate of insoluble matter was formed. This was removed and added to the sodium salts of the primary phthalates. The ether solution was evaporated and the residue dissolved in a mixture of benzene (400 cc.) and alcohol (1600 cc.). A crystalline mass of the paraffin (23 g.) separated on cooling and on evaporation the mother-liquor yielded a further quantity (6 g.) of gummy substance which was also found to be the sodium salt of the secondary alcohol ester.

*Primary alcohols.* The sodium salts of the primary phthalates (12 g.) were hydrolysed by heating with a solution of potassium hydroxide (20 g.) in 95 % alcohol (100 cc.) for 18 hours. The hot solution was filtered and the crystalline mass which separated on cooling was filtered off and dried *in vacuo*. This material was then extracted many times with boiling ether, the ethereal solution filtered, and the solvent removed by distillation. The residue was taken up in petroleum (400 cc. B.P. 40–60°) and boiled with charcoal. From the clarified solution the primary alcohols separated as a white crystalline product (5.7 g.). On repeated crystallisation from various solvents, fractions, varying in M.P. from 80.7° to 82.2°, were obtained together with a small amount (0.9 g.) of crude paraffin, M.P. 62°. The discussion showing that these fractions represented mixed alcohols has already been given, p. 2099.

To obtain further evidence as to the constituent alcohols present in these mixtures they were all united and the combined material (5.0 g.) reduced *via* the iodide, to the corresponding paraffin (4.0 g.) M.P. 61–61.5°. This material was submitted to a prolonged fractionation by means of light petroleum (B.P. 40–60°) in the manner described later for the actual apple paraffin. About 250 samples were obtained during this operation, and there were finally obtained 22 samples weighing in all 1.5 g., whose melting-points and crystal spacings are given in Table I and have been fully discussed in the Introduction.

*Secondary alcohols.* The crude sodium salt (7.2 g.) from the first treatment with phthalic anhydride was heated with potassium hydroxide (10 g.) in alcohol (75 cc.) for 18 hours. The hot solution was filtered and the crystalline solid which separated on cooling was collected and recrystallised from petroleum (B.P. 40–60°). The product, a white crystalline solid (2.4 g.) melted at 80.7°, while the mother-liquor yielded 0.6 g. of material, M.P. 64°, which was added to the crude hydrocarbon fraction described later. The sodium salt (6 g.)

from the second phthalic anhydride treatment was saponified in the same way, and gave a white crystalline substance (2.9 g.), m.p. 80.9–81.2°, together with an impure fraction (0.65 g.) which was added to the crude paraffin fraction. The prolonged treatment with alcoholic potassium hydroxide which we used in this work to saponify the phthalates of the primary and secondary alcohols caused considerable decomposition and darkening of the solution. More recently an alternative method of hydrolysis with sodium ethoxide has been employed which gives a much better recovery of the alcohol. The ester (1 g.) dissolved in benzene (20 cc.) is heated to boiling with alcohol (10 cc.) in which sodium (0.7 g.) has been dissolved. A precipitate of sodium phthalate readily forms and after heating for 5 minutes the solution is kept for 2 hours. Water is then added and the benzene layer evaporated to dryness. Extraction of the residue by petroleum (b.p. < 40°) yields the carbinol as a white crystalline powder (0.7 g.). The two high-melting products from the soluble sodium salts mentioned above gave no depression in m.p. when mixed and appeared to be identical. To make absolutely certain that they were not still contaminated with primary alcohol they were mixed and again put through the phthalate treatment. A small precipitate of insoluble sodium primary alcohol esters was obtained. The secondary alcohol recovered by saponification (3.7 g.), m.p. 80.5–81.5°, was recrystallised from carbon disulphide, acetone and light petroleum, and was finally obtained in long white needles, m.p. 81.9–82.2°. The identification of this alcohol as *d*-10-nonacosanol has been described in some detail in the Introduction. (Found: C, 82.0; H, 14.3. C<sub>29</sub>H<sub>60</sub>O requires C, 82.0; H, 14.2 %.) The mother-liquors after this fractionation were collected and the residue from them crystallised from a small amount of hot acetone. The material (1.25 g.), m.p. 73°, was again fractionated. It yielded 0.46 g. of alcohol, m.p. 79°, which was an impure specimen of *d*-10-nonacosanol and a series of paraffin fractions which melted between 60 and 67°. No evidence for the presence of any other secondary alcohol, for instance one with 27 carbon atoms corresponding to *n*-heptacosane, was found.

The hydrogen phthalate of the alcohol (4.85 g.) after purification was recrystallised from alcohol (charcoal) and was obtained as a white crystalline powder (5.25 g.), m.p. 54.5–54.7°. (Found: C, 77.1; H, 11.3. C<sub>37</sub>H<sub>65</sub>O<sub>4</sub> requires C, 77.4; H, 11.4 %.) For a 20 % solution in chloroform the observed rotation was  $\alpha_{5461}^{17.5} + 0.25^\circ$ , whence  $[\alpha]_{5461}^{17.5} = + 0.62^\circ$ .

*d*-10-Nonacosanol acetate was prepared in the usual way: m.p. 44.5–45°.

*Paraffin fraction.* The crude material was crystallised from benzene-alcohol (23 g.), m.p. 62.8–63.2°. (Found: C, 85.1; H, 14.7. C<sub>29</sub>H<sub>60</sub> requires C, 85.2; H, 14.8 %.) The attempt in previous batches to obtain a pure paraffin from such a product by treatment with sulphuric acid has been described in the Introduction. To fractionate the constituent paraffins the material was stirred for 2–3 minutes at room temperature with petroleum (b.p. 40–60°) in the proportion of 10 cc. of solvent per g. of material. The solvent was filtered off, reduced in volume, and treated with warm acetone (2 : 1). The paraffin

crystallised out and was removed by filtration. This process was repeated until no more of the original material appeared to go readily into the cold solvent. In the present case there was a residue (3.7 g.) from which we were able to obtain no definite substance. Until the possible effect of saponification and treatment with phthalic anhydride on olefines has been investigated we are not examining this material further. The many fractions of paraffins obtained in this way showed a gradual rise in m.p. Each fraction was then further fractionated as follows. The material was completely dissolved by stirring at room temperature in 100 volumes of petroleum (b.p. 40–60°). The solution was then placed in an ice-chest. As it cooled the paraffin crystallised out and by using a cooled suction funnel it was possible to obtain about six successive crops of material. These were found to show a successive decrease in m.p. By dovetailing these two methods of fractionation there was obtained fairly readily a series of 10 fractions melting between 59 and 64°. These were then treated with sulphuric acid at 130° until no further charring occurred. The upper and lower fractions showed considerable charring, the middle ones practically none at all. After this treatment the products were again fractionated and there was finally obtained after some 200 fractionations in all a series of products whose melting-points and crystal spacings (Table I) have been discussed fully in the Introduction.

*Fatty acids.* The amount of fatty acids obtained on saponification of the original crude wax was very small. On decomposition of the sodium salts in the usual way after complete extraction of the unsaponifiable material there were obtained some 5 g. of crude fatty acid. The material was deep green and was obviously very impure, and to obtain a colourless product it had to be boiled many times with alcohol and charcoal. By fractional crystallisation there were then obtained (1) 0.65 g. of fatty acid, m.p. 82.2–82.8°, mol. wt. by titration, 470, clearly the usual mixture of high fatty acids of mean carbon content 28–30 found in many plant waxes, and (2) 2 g. of white crystalline fatty acid, m.p. 55°. These fatty acids may have been present as wax esters with long-chain alcohols or they may have been derived from the residual amount of glycerides present in the crude wax. The amount found, however, shows clearly that the major part of the alcohol present in the wax is free. In some earlier experiments the crude wax was fractionated by means of hot acetone. From a batch of 10 g. there was obtained in one case, 0.3 g. of material almost insoluble in boiling acetone which could be recrystallised from hot ethyl acetate. It melted at 79–80° and was clearly a true wax corresponding with the long-chain primary alcohol and the higher fatty acids which were afterwards isolated. (Found: C, 82.4; H, 13.8.  $C_{28}H_{57}O.O.C_{27}H_{54}$  requires C, 82.4; H, 13.7 %.)

## SUMMARY.

The wax fraction of apple peel has been used to elaborate methods for separating the paraffins, ketones, primary alcohols and secondary alcohols which occur in many plant waxes.

The method of separation adopted is based on the solubility in ether and alcohol of the sodium salts of the secondary alcohol phthalates.

The following constituents of apple peel wax have been definitely identified. *n*-nonacosane, *n*-heptacosane, *d*-10-nonacosanol, *n*-hexacosanol, *n*-octacosanol and *n*-triacontanol.

The bearing of these findings on the metabolism of paraffins in the plant is discussed.

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